Cardiovascular and Respiratory Effects of Adenosine Infusion in Man

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A thesis presented for the degree of

Doctor of Medicine

University of Edinburgh

1991

To my parents

ABSTRACT

Diverse effects of the nucleoside adenosine have been reported including stimulation of respiration in man. This thesis discusses the results of studies undertaken to further characterise the respiratory and concomitant cardiovascular effects of infusion of adenosine in man.

In healthy volunteers intravenous infusion of adenosine, but not its first metabolite inosine, caused a dose-related increase in minute ventilation, not due to hypotension or bronchoconstriction, and a fall in end-tidal carbon dioxide tension.

The increase in minute ventilation was largely due to an increase in tidal volume (V_T). Inspiratory duration (T_I) was unchanged but expiratory duration was slightly reduced. Mean inspiratory flow (V_T/T_I) was increased as was functional residual capacity (FRC). These changes are similar to those that have been reported during equivalent ventilatory stimulation by hypoxia and hypercapnia.

Adenosine caused various symptoms including discomfort in the epigastrium, chest and jaw, flushing, dyspnoea and paraesthesiae, which limited the maximum dose.

Aminophylline antagonised the adenosine-induced increases in ventilation, heart rate and systolic blood pressure and reduced the symptoms, suggesting that these effects are mediated by cell-surface receptors. The venous plasma concentration of adenosine only increased at the higher maximum dose possible after aminophylline, consistent with the very short half-life of adenosine as determined *in vitro*.

Three studies were performed in patients undergoing diagnostic cardiac catheterisation. In one study infusion of adenosine into the aorta proximal to the head and neck vessels caused respiratory stimulation whereas infusion into the upper descending thoracic aorta did not, although chest and epigastric discomfort were more

common. Since in animals adenosine depresses respiration within the central nervous system, this study supports the suggestion that adenosine may stimulate respiration by an action within the carotid bodies. Furthermore it suggests that the respiratory stimulation is not primarily due to symptoms caused by adenosine.

In a second study the mean plasma adenosine concentration in the upper descending thoracic aorta increased from 0.07 μM at baseline to 1.2 μM during iv infusion at the maximum dose. Since micromolar concentrations may be achieved in tissues during hypoxia, due to hydrolysis of adenine nucleotides, these results are consistent with the suggestion that adenosine might participate in the ventilatory response to hypoxia. An unexpected finding was that respiration increased in 3 of the 7 patients before the adenosine concentration did. Since adenosine has a half-life of only a few seconds this finding is consistent with a more proximal effect of adenosine, e.g. within the lungs.

In a third study, which investigated its haemodynamic effects, adenosine increased cardiac output and caused pulmonary and systemic vasodilation. The pulmonary vasodilation first developed at a dose which did not affect systemic vascular resistance suggesting that at low dose ($\leq$ 60 μg/kg/min) adenosine might be useful as a selective pulmonary vasodilator. Higher doses produced an increase in left ventricular end-diastolic pressure of uncertain cause.

Adenosine caused variable effects in 6 patients with chronic hypoxia due to obstructive airways disease. Four showed a slight increase in minute ventilation and improvement in arterial blood gas tensions. None developed worse spirometry but all showed an increase in FRC which was greatest in 2 patients whose tidal volume and arterial oxygen tension fell, suggesting that the changes in lung volume were disadvantageous.

ACKNOWLEDGEMENTS AND DECLARATION

The studies presented in this thesis were performed between July, 1985 and May, 1987 while I was a research fellow in the Departments of Pharmacology and Therapeutics, University of Wales College of Medicine and Respiratory Medicine, Llandough Hospital. I am deeply grateful to Professor Philip Routledge and Dr. A. Peter Smith, my supervisors from those respective departments, for their help, guidance and encouragement throughout. I am also indebted to Dr. Andrew Watt, whose original observations generated many of the questions investigated in this thesis, for his practical help with many of the studies. The study described in Chapter 4 was performed in collaboration with Dr. Watt. He has also given an account of it in his M.D. thesis, Cardiac and Respiratory Effects of Adenosine in Man (Aberdeen University. 1986).

I am also thankful to Dr. Alan Fraser and Professor Andrew Henderson for help with the study described in Chapter 7 and to Dr. Martin Ebejer for help with the study described in Chapter 8. Drs. Liam Penny and Andrew Newby kindly provided details of the adenosine assay which they had developed. Dr. Derek Buss and Mr. Dick Weston and their staff, from the Department of Pharmacology and Therapeutics, and Mr. Kelvin Houston, Mr. Mike Saunders and their staff from the Lung Function Laboratory, Llandough Hospital, willingly gave considerable expert technical assistance. I am also grateful to the staff of the Cardiology Departments of the University Hospital of Wales and Llandough Hospital for their technical assistance during those studies which required invasive haemodynamic monitoring. Cardiac catheters were inserted by Drs. Fraser and Watt. Dr. K. Stühmeyer kindly provided translations of some papers written in German. I acknowledge the helpful comments of Dr. Roger Newcombe of the Department of Statistics and Computing, University of Wales College of Medicine and Dr. John Matthews of the Division of Medical Statistics,

University of Newcastle upon Tyne. I am beholden to the many patients and volunteers who consented to participate in the studies.

Lastly I thank my wife and children for their support and forbearance during this phase of our lives.

I declare that all of the studies described in this thesis were performed by myself with the practical assistance of the people indicated above. The medical practioners mentioned made helpful contributions to the interpretation of the data. The thesis was composed by myself.

Peter G. Reid January, 1991.

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ABBREVIATIONS

In addition to standard abbreviations for commonly used units, the following abbreviations were used in this thesis. Some abbreviations used only once, or in figures or tables, are defined where used.

ANOVA	repeated measures analysis of variance
ADP	adenosine diphosphate
AMP	adenosine monophosphate
ATP	adenosine triphosphate
BP	blood pressure
CAD	coronary artery disease
CI	cardiac index
CO	cardiac output
ECG	electrocardiogram
EHNA	erythro-9-(2-hydroxy-3-nonyl) adenine
$FEF_{25-75\%}$	forced expiratory flow rate between 25 and 75% of forced vital capacity
FEV_1	forced expiratory volume in 1 second
f_R	respiratory rate
FRC	functional residual capacity
FVC	forced vital capacity
HPLC	high performance liquid chromatography
HR	heart rate
NECA	5'-N-ethylcarboxamidoadenosine
PaO_2	arterial oxygen tension
$PaCO_2$	arterial carbon dioxide tension
PAP	pulmonary artery pressure
PCWP	pulmonary capillary wedge pressure
PEFR	peak expiratory flow rate
$PETCO_2$	end-tidal PCO_2
PVR	pulmonary vascular resistance
RAP	right atrial pressure
RIP	respiratory inductance plethysmograph
R-PIA	N^6-R-phenylisopropyladenosine
SD	standard deviation
SEM	standard error of the mean

SVI	stroke volume index
SVR	systemic vascular resistance
T_E	expiratory duration
T_I	inspiratory duration
T_{TOT}	total breath duration
V	minute ventilation
VO_2	total oxygen consumption
V_T	tidal volume

CHAPTER 1 - INTRODUCTION

1.1 - GENERAL INTRODUCTION

Adenosine is a purine nucleoside whose biological effects were first described in 1929 by Drury and Szent-Györgyi. Since those observations there has been much research into the biochemistry, physiology and pharmacology of adenosine. This has been especially true over the last three decades following the suggestion by Berne et al. (1963) that adenosine might be a mediator of the metabolic regulation of coronary blood flow. In a recent review it was estimated that over 20,000 studies of the formation and effects of adenosine have been performed since the work of Drury and Szent-Györgyi (Sollevi, 1986).

This thesis contains results of studies undertaken to investigate the cardiovascular and respiratory effects of the infusion of adenosine in man. The starting point for this work was the observation by Watt and Routledge (1985) that bolus injections of adenosine cause transient powerful stimulation of respiration in man. The questions raised by that observation which this thesis seeks to answer are summarised in the final section of the introduction. In the preceding sections published articles on the biochemistry and effects of adenosine are reviewed. Emphasis is given to previous studies directly relevant to the present work. Discussion of some of the more recent papers is left to later chapters.

1.2 - BIOCHEMICAL ASPECTS

Adenosine is a nucleoside of molecular weight 267.2 formed by the condensation of the purine adenine and the C5 sugar ribose (Fig. 1.1). It is produced in and metabolised by most animal tissues. Some important pathways of adenosine metabolism are shown in Fig. 1.2. Adenosine is a precursor and degradation product of the nucleotides adenosine 5'-monophosphate, 5'-diphosphate and 5'-triphosphate (AMP, ADP and ATP) (Arch & Newsholme, 1978).

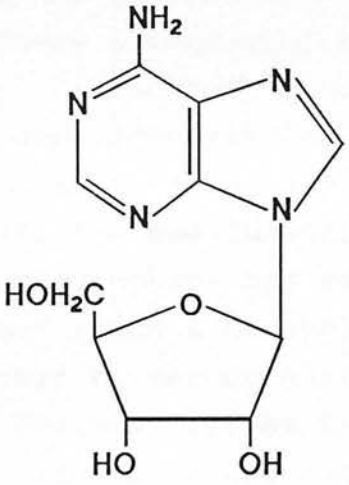

Fig. 1.1 - Chemical structure of adenosine.

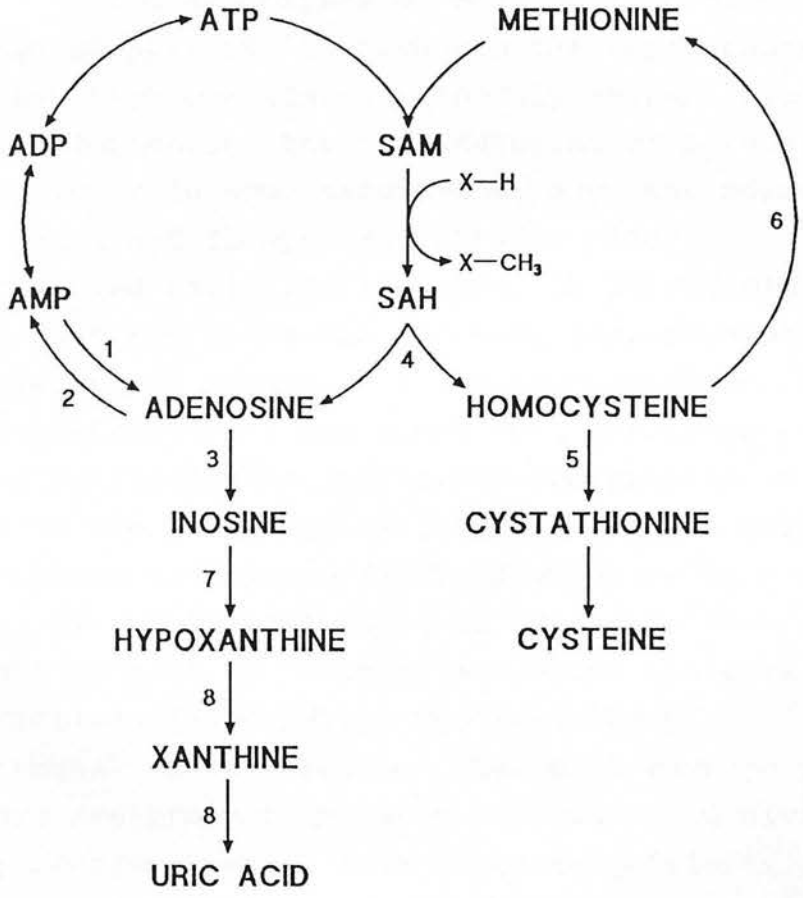

Fig. 1.2 - Important pathways of adenosine metabolism.

SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine; 1, 5'-nucleotidase; 2,adenosine kinase; 3, adenosine deaminase; 4, S-adenosylhomocysteine hydrolase; 5, cystathionine-ß-synthase; 6, betaine homocysteine methyltransferase; 7, purine nucleoside phosphorylase; 8, xanthine oxidase. (After: Lloyd & Schrader, 1987).

There are many reports of increased adenosine liberation in tissues when energy (or oxygen) requirements are increased or supply is reduced, situations which usually result in greater dephosphorylation of adenine nucleotides (e.g. Olsson, 1970; Rubio et al., 1975; Mentzer et al., 1975 and see Chapter 6). Following release in this way adenosine has been considered to act as a "local hormone" (Arch & Newsholme, 1978). Since many of its actions appear to reduce energy demand or increase energy supply and thereby redress the balance between supply and demand, adenosine has also been described as a "retaliatory metabolite" (Newby, 1984).

Adenosine is also formed in the "transmethylation pathway" by the hydrolysis of S-adenosylhomocysteine (Schrader et al., 1981). Although the dephosphorylation of AMP has been considered primarily responsible for adenosine formation, the transmethylation pathway may be more important in some situations, e.g. the normoxic guinea pig heart (Lloyd and Schrader, 1987).

Sattin and Rall (1970) showed, in guinea pig cerebral cortex, that adenosine can increase the intracellular concentration of adenosine 3',5'-monophosphate (cyclic AMP). Subsequently it was shown that adenosine can also decrease intracellular cyclic AMP concentrations (Fain et al., 1972; van Calker et al., 1978; Fain and Malbon, 1979). Haslam and Lynham (1972) showed, using platelets, that at low concentrations ($\leq$ 25 μM) adenosine stimulated adenylate cyclase in isolated membranes while at higher concentrations (>100 μM) it was inhibitory.

Burnstock (1978) proposed that cell-surface purine receptors are present in various tissues and divided them into P_1 receptors which have a greater affinity for adenosine and P_2 receptors which have a greater affinity for ATP. Van Calker et al. (1979) provided evidence of two classes of the adenosine receptors which they termed A_1 and A_2. Their A_1 receptor mediated inhibition of adenylate cyclase at submicromolar concentrations of adenosine, whereas the A_2 receptor mediated activation of

adenylate cyclase at concentrations of adenosine above micromolar.

Londos et al. (1980) used the nomenclature R_i and R_a to denote the adenosine receptors mediating respectively the inhibition and the activation of adenylate cyclase. The same group had also described an intracellular site inhibitory to adenylate cyclase, termed the "P site" (not to be confused with Burnstock's P_1 and P_2 receptors) (Londos & Wolff, 1977). Presently the A_1, A_2 notation is used most widely to describe the cell-surface receptors, although additional classes and sub-classes have been proposed (Bruns, 1986).

Van Calker et al. (1979) also showed that the order of potency of various adenosine analogues differs at the two types of cell-surface receptor. The order of potency of such analogues has proved to be a useful tool for classifying receptors involved in particular physiological processes as either A_1 or A_2 (Daly et al., 1987), e.g. at A_1 receptors N^6-R-phenylisopropyladenosine (R-PIA) is more potent than 5'-N-ethylcarboxamido-adenosine (NECA) while at A_2 receptors the reverse is true. This method is now preferred to classification according to effects on adenylate cyclase since receptor mediated effects of adenosine may in some situations involve different effector systems. Adenosine receptors are distributed widely throughout the body and mediate diverse effects (Table 1.1).

Methylxanthines such as caffeine and theophylline have been shown to act as competitive antagonists of those actions of adenosine which are now known to be mediated by cell surface receptors. Nichols & Walaszek (1963) first showed that caffeine antagonised the hypotensive effect of adenosine and related nucleotides in various species. Methylxanthines antagonised the adenosine-induced increase in cyclic AMP in guinea pig cerebral cortex observed by Sattin & Rall (1970) and the activation of platelet adenylate cyclase reported by Haslam and Lynham (1972). Subsequently numerous studies

Table 1.1 - Adenosine receptors: distribution and function.

Tissue or cell type	Effects mediated
A_1 (R_I) adenosine receptors	
Brain	Sedation
	Inhibition of neurotransmitter release
Adipose tissue	Inhibition of lipolysis
Heart	Suppression of contractility
	Inhibition of sinoatrial node
	Inhibition of atrioventricular node
A_2 (R_A) adenosine receptors	
Brain	Modulation of neurotransmission
Carotid body	Chemoexcitation
Platelet	Inhibition of aggregation
Smooth muscle	Relaxation
Kidney	Vasoconstriction
Liver	Stimulation of gluconeogenesis
Neutrophils	Inhibition of superoxide release

After: Clarke & Coupe, 1989.

have confirmed similar effects in a wide range of tissues. Methylxanthines have previously been considered to exert their biological effects largely by inhibiting intracellular phosphodiesterases which hydrolyse cyclic AMP. However higher concentrations are required to inhibit phosphodiesterases (50% inhibition by theophylline at approximately 400 to 1000 μM) than are required to antagonise the effects of adenosine (half maximal antagonism at approximately 4 to 60 μM: 1 to 10

μg/ml) (Butcher & Sutherland, 1962; Fredholm et al., 1976; Smellie et al., 1979; Daly, 1983). Adenosine antagonism has therefore been proposed as a mechanism of the pharmacological actions of the methylxanthines (Fredholm, 1980; Snyder et al., 1981; Daly et al., 1981). Chronic consumption of caffeine or theophylline results in potentiation of the pharmacological effects of adenosine and upregulation of receptor number in animals (von Borstel et al., 1983; Wu et al., 1989). Xanthines have no effect on the intracellular P-site-mediated inhibition of adenylate cyclase (Daly, 1982).

There are avid uptake mechanisms for adenosine in cell membranes and these have considerable importance for the actions of adenosine since they serve to remove the nucleoside from its site of action at cell-surface receptors (Huang & Daly, 1974). After transport into human red cells adenosine is rapidly metabolised (Schrader et al., 1972; Plageman et al., 1985). At extracellular concentrations of less than 1 to 3 μM adenosine is predominantly incorporated into adenine nucleotides whereas at concentrations greater than 6 to 10 μM adenosine kinase is inhibited and most of the adenosine transported into cells is deaminated (Plageman et al., 1985). Due to its rapid uptake into blood cells adenosine has a very short half-life. Klabunde (1983) estimated the half-life in human blood at 37° C to be less than 10s. Recently Möser et al. (1989) showed that at concentrations of 1 to 5 μM there is net removal of adenosine from human plasma with a half-life of approximately 1.5 s.

Adenosine transport into cells is inhibited by a number of compounds including dipyridamole (Koss et al., 1962; Bunag et al., 1964; Huang & Daly, 1974; Klabunde, 1983). By increasing the extracellular concentration such compounds potentiate those effects of adenosine which are mediated by cell-surface receptors and have proved useful tools for investigating such effects.

Adenosine may also be metabolised extracellularly,

e.g. by deamination, but this seems to be quantitatively less important than cellular uptake and intracellular metabolism (Arch & Newsholme, 1978).

1.3 - BIOLOGICAL EFFECTS OF ADENOSINE

1.3.1 - RESPIRATORY EFFECTS

a) - Effects on the control of breathing

Although some early investigators had observed that administration of adenosine or the related nucleotide ATP could affect breathing in various ways in a variety of species including man (Drury & Szent-Györgyi, 1929; Emmelin & Feldberg, 1948; Green & Stoner, 1950) it was in the 1980s that interest centred on the action of adenosine as a respiratory depressant. Several workers showed that administration within the central nervous system of adenosine (Kattwinkel & Darnall, 1982) and its long-acting analogues R-PIA (Hedner et al., 1982, Lagercrantz et al., 1984, Eldridge et al., 1984), 2-chloroadenosine (Kattwinkel & Darnall, 1982, Mueller et al., 1982, Wessberg et al., 1985) N^6-cyclohexyladenosine and NECA (Wessberg et al., 1985) could depress respiration in a variety of species. This was observed in both anaesthetised and naturally sleeping animals. Both depth and rate of respiration were affected. Inspiratory drive was decreased and expiratory time was prolonged (Wessberg et al., 1985).

Similar effects have been shown with peripheral (intra-peritoneal or intravenous) administration of analogues, but much larger doses are required consistent with a central site of action (Lagercrantz et al., 1984, Wessberg et al., 1985). Such effects persist despite vagotomy and glomectomy, suggesting that pulmonary and peripheral chemoreceptor afferents are not required. Lagercrantz et al. (1984) observed respiratory depression in decerebrate animals suggesting that it is not mediated at a suprapontine level. Adenosine has been found to

reduce not only resting ventilation but also the ventilatory response to hypercapnia (Wessberg et al., 1984).

The effects on respiration of R-PIA in neonatal animals are more marked in younger than older animals (Lagercrantz et al., 1984) and have been found to correlate with the affinity of R-PIA receptors in whole brains (Runold et al., 1986).

Respiratory depression by adenosine and its analogues is antagonised by theophylline (Lagercrantz et al., 1984; Eldridge et al., 1985) and aminophylline (Watt et al., 1987a), but not by enprofylline, a xanthine without adenosine receptor-blocking activity (Wessberg et al., 1985; Darnall et al., 1985). Millhorn et al. (1984) reported that theophylline reduced the magnitude of acute hypoxic and the duration of post-hypoxic depression of phrenic nerve activity in anaesthetised, paralysed, vagotomised and glomectomised cats. They suggested that an increased concentration of adenosine within the brain caused the long-lasting depression of respiration following hypoxia and was partially responsible for the acute effects. This is supported by the finding in a variety of species that the adenosine concentration in the brain is increased by hypoxia. Furthermore methylxanthines stimulate breathing (see Chapter 5) suggesting that adenosine might normally exert tonic modulation of respiration. Methylxanthines have been found to be effective treatment for neonatal apnoea (Kuzembo & Paala, 1973) and it has been suggested that this abnormality might therefore be mediated by a central respiratory depressant effect of adenosine (Lagercrantz et al., 1984).

Despite such evidence for a respiratory depressant effect of adenosine and its analogues, several workers had previously noted that adenosine and related compounds can stimulate breathing. Drury and Szent-Györgyi (1929) observed that "panting respiration" might occur after subcutaneous injection of adenosine in guinea pigs. In

one patient after an intravenous bolus of adenosine Honey et al. (1930) observed that:

"the breathing became deeper and more rapid, the patient became restless, felt breathless, and experienced a sense of constriction of the chest."

In a further patient given a larger dose:

"the breathing became faster and more shallow."

Rowe et al. (1962) observed increased minute ventilation and CO_2 elimination in dogs given intravenous ATP or adenosine. Gustafsson (1981) observed that infusion of adenosine into the left ventricle of anaesthetised rabbits sometimes induced "forceful spontaneous respiration" despite vagotomy, suggesting that the effect was not mediated by pulmonary afferents.

ATP is rapidly hydrolysed in vivo thus many of its actions may be due to liberated adenosine. Emmelin and Feldberg (1948) reported variable responses to intravenous ATP, with some animals showing "strong hyperventilation". Green and Stoner (1950) found that while intravenous ATP and to a lesser degree adenosine usually slowed respiration in animals acceleration of breathing often preceded this and was sometimes the only response. Davies et al. (1951) studied the effects of ATP in 28 patients with a variety of conditions and found that intravenous injection caused a marked increase in the depth of respiration with little change in rate. A similar but smaller response was seen with intra-arterial injection. These changes were accompanied by striking subjective responses including "a peculiar, painless sensation in the chest", a feeling of suffocation and apprehension.

In 1985 Watt and Routledge published the first detailed examination of respiratory stimulation by adenosine. They found that intravenous bolus injections

in healthy volunteers caused a dose-related increase in ventilation, mostly due to increased depth of breathing. The effect was transient, lasting approximately 20s, and began 15 to 20s after the injection. Adenosine also caused a transient sinus bradycardia followed by a brief sinus tachycardia. Watt and Routledge considered that because of the findings of Gustafsson (1981: see above) the respiratory stimulation was unlikely to be secondary to stimulation of pulmonary sensory afferents. Furthermore the evidence for a central depressant effect on respiration of adenosine suggested that a direct stimulant effect within the central nervous system was unlikely. Since the aortic chemoreceptors are unimportant in man (Lugliani et al., 1971) Watt and Routledge considered that the carotid bodies were the most likely site of action of adenosine. They also considered this consistent with their observation that respiratory stimulation began up to 2 s after the longest R-R interval. Blood pressure and airway calibre were not measured in the study of Watt and Routledge so contributory effects of hypotension and broncho-constriction, both of which can be caused by adenosine (see below), could not be excluded by their work.

Evidence for an action of adenosine within the carotid bodies had previously been provided by McQueen and Ribeiro (1981) who showed that intracarotid injections of adenosine caused a dose-related increase in chemosensory discharges in the carotid sinus nerve of anaesthetised cats. Although initial work showed no antagonism of this effect by theophylline or aminophylline, the adenosine uptake blocker dipyridamole enhanced the effect suggesting an action of adenosine on cell-surface receptors (McQueen & Ribeiro, 1983). This suggestion was supported by the finding that adenosine analogues active at such sites caused chemoreceptor activation, whereas an agonist at the intracellular receptor, the P-site (Londos & Wolff, 1977), was without effect (McQueen & Ribeiro, 1983). These observations, the

finding that ATP, a precursor of adenosine, is stored in the carotid body (Böck, 1980) and the reports that adenosine production is increased in a number of organs during hypoxia (see above and Chapter 6), led Watt and Routledge (1985) to suggest that adenosine may be a mediator within the carotid body of the ventilatory response to hypoxia. Subsequent studies relating to this topic will be discussed in later chapters.

b) - Effects on the airways

It has been known since the work of Bennett and Drury (1931) that adenosine can relax tracheal smooth muscle. While some recent workers have observed that adenosine may cause contraction of tracheal muscle at resting tone (Fredholm et al., 1979; Advenier, 1982) several groups have confirmed that adenosine can cause relaxation when the tracheal muscle is preconstricted or has a high initial resting tone (Coleman & Levy, 1974). The relaxation is enhanced by adenosine uptake-blockers such as dipyridamole (Coleman, 1976; Farmer & Farrar, 1976) and antagonised by methylxanthines (Karlsson et al., 1982).

While investigating whether adenosine may be similarly effective at relaxing bronchial smooth muscle, Cushley et al. (1983a) were surprised to observe that inhaled adenosine caused bronchoconstriction in asthmatic subjects (Holgate et al., 1987). This effect reached a peak within 1 to 3 minutes and abated slowly over the following hour. A similar effect was not seen in normal subjects. The related nucleoside guanosine was inactive suggesting that a non-specific irritant effect was not implicated (Cushley et al., 1983a) and indices of non-specific bronchial responsiveness, ie) to methacholine and histamine, were only weakly correlated with the response to adenosine (Mann et al., 1986; Cushley & Holgate, 1985).

Subsequently the principle breakdown product of adenosine, inosine, was found to be inactive but the

nucleotides, AMP and ADP, which can be rapidly hydrolysed to adenosine in vivo, caused similar effects (Cushley et al., 1983b; Mann et al., 1986). Bronchoconstriction by adenosine was later shown to be specifically antagonised by theophylline (Cushley et al., 1984, Mann & Holgate, 1985) and enhanced by dipyridamole (Cushley et al., 1985), suggesting that this effect of adenosine is mediated by cell-surface receptors. Mann et al. (1985) later provided evidence suggesting that the effect is not mediated by stimulation of cholinergic reflexes or reduced β_2-adrenoceptor responsiveness of the airways.

The mechanism of the bronchoconstrictor effect of adenosine remains to be fully elucidated but several strands of evidence suggest that potentiation of mast cell mediator release is implicated (Cushley & Holgate, 1985; Holgate et al., 1987).

Whether adenosine has a rôle in the pathogenesis of asthma remains unclear. Marquardt et al. (1984) reported release of adenosine by IgE-dependent activation of murine bone marrow-derived mast cells. Mann et al. (1986) showed an increase in plasma venous concentration of adenosine following both antigen and methacholine bronchial provocation in asthma, but since adenosine has a very short half-life it is uncertain whether the increase was due to adenosine release from the lungs.

Although inhaled adenosine was used in most initial studies of its airway effects, Pauwels and van Der Straeten (1986) reported that intravenous adenosine caused bronchoconstriction in inbred rat strains. However they noted marked differences in response between rat strains and also found that inosine was active. Bilateral vagotomy did not alter the response but atropine caused slight inhibition. Theophylline, but not enprofylline, antagonised the response but no enhancement was seen with dipyridamole. The order of potency of various adenosine analogues led the authors to suggest that the effects of adenosine were mediated by A_2 adenosine receptors. The type of anaesthetic used affected the response in some

strains so the relevance of their findings to those in conscious humans is uncertain. Taviot et al. (1986) reported a case of severe bronchospasm in an asthmatic patient approximately 2 min after he was given intravenous ATP, which is rapidly degraded to adenosine, to treat a supraventricular tachycardia. However at the same time as the injection the patient had performed various vagal manoeuvres which might have contributed to his bronchoconstriction. Results of studies specifically examining the airway effects of intravenous adenosine will be discussed in later chapters.

c) - Effects on the diaphragm

While various workers have suggested that the enhancement of diaphragmatic contractility produced by methylxanthines such as theophylline and caffeine might be due to antagonism of endogenous adenosine (Murciano et al., 1984; Aubier et al., 1983), Supinski et al. (1986) found that adenosine had no effect on the force of contraction of isolated guinea pig diaphragm, nor did it affect the increase in diaphragmatic tension produced by theophylline.

1.3.2 - CARDIOVASCULAR EFFECTS

a) - Vascular effects

Drury & Szent-Györgyi (1929) first reported that adenosine increased coronary flow in a dog heart-lung preparation and reduced arterial blood pressure in dogs and cats. They concluded that adenosine is a coronary and systemic vasodilator. Subsequently it has been shown that adenosine causes potent vasodilation in many tissues including the heart (Wedd, 1931; Watt et al., 1987c), skeletal muscle (Dobson et al., 1971), brain (Berne et al., 1974), adipose tissue (Sollevi & Fredholm, 1981) and gut (Granger & Norris, 1980). A different response has been seen in the kidney (see below) and hepatic veins where adenosine causes vasoconstriction.

In 1963 Berne proposed that adenosine may be a mediator of metabolic autoregulation of coronary blood flow. To date this hypothesis is unproven and evidence has been presented from various animal models which suggests that an adenosine-mediated effect is not necessary for the functional response to physiological stimuli such as increased work (e.g. Downing & Chen, 1986; Bache et al., 1988).

The mechanisms of adenosine-induced vascular smooth muscle relaxation remain to be fully elucidated. An endothelium-dependent mechanism has been demonstrated (Nees et al., 1987). However adenosine can also relax vascular smooth muscle directly (Herlihy et al., 1976). Alterations in cyclic AMP do not seem to be implicated in this response (Herlihy et al., 1976) but Kurtz (1987) showed that adenosine can stimulate guanylate cyclase, via an A_1 type receptor, and increase intracellular cyclic guanosine monophosphate, a known mediator of vascular smooth muscle relaxation. Fenton et al. (1982) suggested that adenosine causes smooth muscle relaxation by impairing calcium uptake during stimulation.

As a coronary vasodilator adenosine has shown promise as an alternative to dipyridamole in the diagnosis of coronary disease by stress Thallium-201 myocardial scintigraphy (Verani et al., 1990) or 2-dimensional echocardiography (Trakhtenbroit et al., 1990). It can also dilate coronary vein grafts (Torsell et al., 1985).

Until recently the haemodynamic effects of the systemic administration of adenosine had been studied in detail only in laboratory animals. Since they cause systemic vasodilation the potential use of both ATP and adenosine as hypotensive agents during anaesthesia has been investigated in both animals and man (Fukunaga et al., 1982a; Fukunaga et al., 1982b; Fukunaga et al., 1983). ATP may cause unwanted effects (Dedrick et al., 1982; Boarini et al., 1984) and there is evidence that its action as a vasodilator depends on prior breakdown to adenosine (Sollevi et al., 1984a).

The reduction in blood pressure caused by adenosine is stable without either tachyphylaxis or rebound hypertension (Fukunaga et al., 1982a), possibly due to inhibition by adenosine of an increase in renin release (Lagerkranser et al., 1985). Adenosine has proved safe during administration at hypotensive doses for at least 30 minutes (Öwall et al., 1987) although in occasional patients ECG changes suggesting myocardial ischaemia have developed (see Chapter 7). Although Sollevi (1986) reported that urine output is markedly reduced during adenosine-induced hypotension, postoperative serum creatinine concentrations were unchanged (Sollevi et al., 1984b).

Studies in anaesthetised animals and man which have examined the responses to systemic administration of adenosine will be discussed in Chapter 7.

At the commencement of the present work little was known about the vascular effects of adenosine in conscious man. DiMarco et al. (1985) did not observe hypotension following bolus injections given to treat arrhythmias but the effects of continuous infusion had not been studied.

b) - Electrophysiological effects

Drury and Szent-Györgyi (1929) first reported slowing of the sinus rate and depression of AV conduction by adenosine in animals. Similar effects were subsequently observed following intravenous boluses in man (Honey et al., 1930; Jezer et al., 1933). More recently adenosine has been shown to be an effective treatment for supraventricular tachycardias in both adults and children (DiMarco et al., 1983; Watt et al., 1986a; Clarke et al., 1987; Till et al., 1989). Since adenosine slows conduction through the atrioventricular node it reduces the ventricular response to atrial tachyarrhythmias such as flutter and fibrillation (DiMarco et al., 1985). Reversal of atropine-resistant atrioventricular block complicating myocardial infarction by the adenosine

antagonist aminophylline suggests that an increased endogenous adenosine concentration might be implicated in the pathogenesis of that arrhythmia (Wesley et al., 1986; Shah et al., 1987).

The only patients with ventricular tachycardia in which adenosine has an antiarrhythmic effect comprise a small subset with exercise-induced arrhythmias and otherwise normal hearts (Lerman et al., 1986; Griffith et al., 1988). Adenosine may therefore be useful for diagnosing the origin of broad complex tachycardias (Griffith et al., 1988; Rankin et al., 1989).

The mechanisms of the electrophysiological effects remain to be fully elucidated (Bellardinelli & Lerman, 1990) but include shortening of the action potential and membrane hyperpolarisation by increasing potassium conductance in the atrium (Bellardinelli et al., 1983) and antagonism of catecholamine-induced inward calcium currents in the ventricle (Isenberg & Belardinelli, 1984).

c) - Effects on myocardial function

Adenosine exerts a strong negative inotropic effect on atrial myocardium (Drury & Szent-Györgyi, 1929; Collis, 1983; Böhm et al., 1985a), has little direct effect on ventricular function (Dobson, 1983) but can antagonise both the release of noradrenaline (Hedqvist & Fredholm, 1979) and the ß-adrenoceptor mediated effects of catecholamines on contractility and metabolism of ventricular muscle (Schrader et al., 1977; Dobson, 1978; Endoh & Yamashita, 1980; Böhm et al., 1985b). It has been suggested that adenosine acts as a negative feedback modulator during stimulation of the ventricular myocardium by catecholamines (Dobson et al., 1986). Whether the action on ventricular contractility is mediated by a reduced intracellular concentration (Dobson, 1983) or by a reduced effect (Böhm et al., 1984) of cyclic AMP remains controversial.

Despite the above effects adenosine infusion has been

shown to improve myocardial recovery following cardioplegia, possibly by enhancing repletion of tissue adenine nucleotide content (Thelin et al., 1989; Bolling et al., 1990).

1.3.3 - OTHER EFFECTS

A wide range of other effects of adenosine has been reported (Watt & Routledge, 1986c; Anonymous, 1985).

In the kidney adenosine infusion may cause transient vasoconstriction, a sustained redistribution in renal blood flow and a sustained reduction in glomerular filtration rate and salt and water excretion (Drury and Szent-Györgyi, 1929; Tagawa & Vander, 1970; Osswald, 1975). The effect on renal blood flow and glomerular filtration correlates with the degree of activation of the renin-angiotensin system (Thurau, 1964; Osswald et al., 1978). Adenosine may inhibit renin release (Tagawa & Vander, 1970; Osswald et al., 1978), an effect which may contribute to its usefulness as a vasodilator (Fukunaga et al., 1982a; Lagerkranser et al., 1985). Katholi et al. (1984) showed that intrarenal infusion of adenosine in the dog causes increased sympathetic activity resulting in increased heart rate, cardiac output and blood pressure. The effects were abolished by renal denervation.

In laboratory animals adenosine and its analogues cause marked behavioural effects such as sedation (Maitre et al., 1974; Dunwiddie & Worth, 1982), reduced locomotor activity (Dunwiddie & Worth, 1982) and induction of sleep (Haulica et al., 1973). Adenosine receptors have been identified throughout the brain (Bruns et al., 1987). Adenosine can modulate neurotransmission by inhibiting spontaneous and evoked neuronal firing and release of neurotransmitters (including acetylcholine, noradrenaline and dopamine) in the central and peripheral nervous system (Ginsborg & Hirst, 1972; Hedqvist & Fredholm, 1976; Michaelis et al., 1979). Adenosine may also be implicated in hypoxic depression of neuronal activity in

the brain (Millhorn et al., 1984) and spinal cord (LLoyd et al., 1988). Since methylxanthines such as caffeine and theophylline are adenosine antagonists their stimulant effects may be due to reversal of the central actions of adenosine (Snyder et al., 1981). Adenosine can suppress ictal activity (Maitre et al., 1974; Dunwiddie & Worth, 1982; Dragunow, 1986) and may be implicated in the actions of some anticonvulsants (Weir et al., 1984).

Adenosine may have a rôle in the causation of pain although some of the evidence appears contradictory. Gourley and Beckner (1973) showed that intraperitonial injection of adenosine, or adenine and its nucleotides, antagonised the analgesic effect of morphine in mice. Furthermore Bleehen and Keele (1977) reported that adenosine caused pain when applied to blister bases in man. However Ho et al. (1973) showed that theophylline, a known adenosine antagonist, inhibited the analgesic effect of morphine in mice and Stone and Perkins (1979) suggested, on the basis of experiments in rats, that endogenous adenosine might mediate the analgesic effect of opiates. Morphine had been shown to enhance adenosine release from rat cerebral cortex (Fredholm & Vernet, 1978; Phillis et al., 1980b) and several groups have subsequently demonstrated anti-nociceptive effects of adenosine and its analogues in various animal models (Snyder et al., 1981; Yarbrough & McGuffin-Clineschmidt, 1981; Ahlijanian et al., 1985).

However more recently Sylvén et al. (1986) observed that intravenous injections of adenosine in normal volunteers provoked chest pain and suggested that adenosine release during ischaemia may mediate the warning symptom of angina. Watt et al. (1987) also reported that in patients with recently active peptic ulcers intravenous injections of adenosine precisely reproduced their pain and Sylvén et al. (1988) showed that injections of adenosine into the brachial artery of volunteers caused pain in the forearm.

The apparent contradictions of studies so far suggest

that adenosine might have an analgesic action within the brain and spinal cord but an algogenic effect on peripheral nerves. The putative rôle of adenosine as a mediator of angina will be discussed further in Chapters 4 and 7.

Adenosine may cause hypothermia (Dunwiddie & Worth, 1982) and various metabolic effects, e.g. antagonism of the effects of catecholamines in the heart (Schrader et al., 1977) and adipose tissue, and inhibition of insulin secretion but enhancement of its action (Schwabe et al., 1974).

Adenosine inhibits platelet aggregation in vitro (Born, 1964; Born et al., 1964; Edlund et al., 1987) and has been shown to reduce platelet consumption during cardiopulmonary bypass (Sollevi et al., 1985). Since the cellular uptake of adenosine is inhibited by dipyridamole, the antiplatelet effect of that drug might be mediated by an increase in the endogenous adenosine concentration.

Adenosine may impair immune functions, e.g. at the high concentrations found in the rare condition adenosine deaminase deficiency adenosine may be toxic for lymphoid cells (Hershfield et al.,1987). Adenosine has been shown to inhibit many activities of neutrophils, e.g. release of oxygen free radicals (Cronstein et al., 1987). Such effects may contribute to the observed reduction by adenosine of reperfusion injury in experimental animals (Olafsson et al., 1987).

1.4 - PROPOSALS FOR A THESIS

The observation by Watt and Routledge (1985) that intravenous bolus injections of adenosine caused marked transient stimulation of respiration in man raised a number of questions:

1) Is the respiratory stimulant effect of adenosine sustained during continuous infusion ?
2) Do haemodynamic changes, e.g. hypotension, contribute?
3) Does the response show tachyphylaxis?
4) Is it dose-dependent?
5) At what concentrations of adenosine are its effects produced?
6) Is the effect produced by adenosine <u>per se</u> or one of its metabolites?
7) Is the response modified by aminophylline, an antagonist at adenosine cell-surface receptors?
8) Is the response potentiated by dipyridamole, an inhibitor of cellular uptake of adenosine?
9) Is the effect mediated by the carotid body?
11) Does intravenous adenosine cause broncho-constriction in normal subjects, patients with asthma or chronic airflow limitation?
12) Does adenosine have a physiological rôle in the control of respiration?
13) Is the response to intravenous adenosine altered in patients with respiratory failure?
14) Does adenosine have a potential therapeutic rôle in respiratory failure?

The work described in this thesis attempts to give answers to some of these questions and also to further pertinent questions raised as the work proceeded.

CHAPTER 2 - METHODS

2.1 - GENERAL INTRODUCTION

This chapter describes the methods and materials used for measuring respiration and plasma adenosine concentrations and the infusion technique employed. It also discusses some general and ethical considerations. Other methods used are described in the relevant chapters.

2.2 - MEASUREMENT OF RESPIRATION

2.2.1 - INTRODUCTION

In all studies described in this thesis the subjects' respiration was measured. Measurement of minute ventilation and its component parts, respiratory frequency and tidal volume, has been widely used for the assessment of drug effects on the control of breathing (Jordan, 1982). Methods which employ either a mouth piece and nose clips or a closely fitting face mask have commonly been used but have several disadvantages. Firstly such methods can alter breathing pattern, mainly by increasing tidal volume (Gilbert et al., 1972; Askanazi et al., 1980). Secondly it is harder for subjects to speak while using a mouth-piece or face mask, making it difficult for them to report adverse effects. Thirdly such apparatus may be uncomfortable and therefore not tolerated for long periods. For these reasons less invasive methods were employed in this work. Two methods were used, both of which rested upon the assumption that volume changes of the lungs could be estimated from changes in the external dimensions of the torso. These methods are now discussed.

2.2.2 - RESPIRATION TRANSDUCER

a) - Description and Use

The initial equipment available was a respiration transducer (Type 4320. Lectromed Limited, St. Peter,

Jersey) which employs an elasticated band connected to 2 strain gauges which form part of a Wheatstone bridge network. Changes in volume and therefore diameter of the thorax cause stretch of the elasticated band which, by Hooke's law, should cause a linearly related increase in tension. Changes in tension applied to the strain gauges alter the relative potential difference across the 2 halves of the bridge network. The voltage difference between no load and a load of approximately 0.45 kg applied across the transducer is between 1 mV and 2 mV when an excitation voltage of 8 V is used.

The elasticated band of the transducer was secured around the chest approximately at the level of the xiphisternum where respiratory excursions were maximum. The signal from the transducer was amplified and recorded using a chart recorder (Ormed MX 216). Respiratory frequency and tidal volume were determined manually from the recordings and used to calculate minute ventilation.

b) - Volume Calibration

For each subject approximately 10 separate breaths over a range of tidal volumes were recorded, in the posture maintained throughout the study, simultaneously using the respiration transducer and either a Vitalograph spirometer (Vitalograph Limited, Buckingham, England) or a Pocket spirometer (Micro Medical Instruments, Strood, Kent) which has been validated previously (Chowienczyk & Lawson, 1982). The recorded tidal volumes and transducer signal deflections for each subject were plotted and a regression line through the origin was fitted by the method of least squares to obtain a calibration curve. An example is shown in Fig. 2.1.

c) - Reliability

One indication of the precision of the technique is provided by the scatter of values about the regression lines obtained during volume calibration. In the study described in Chapter 4 a mean of 35 (SD: 20)% of breath

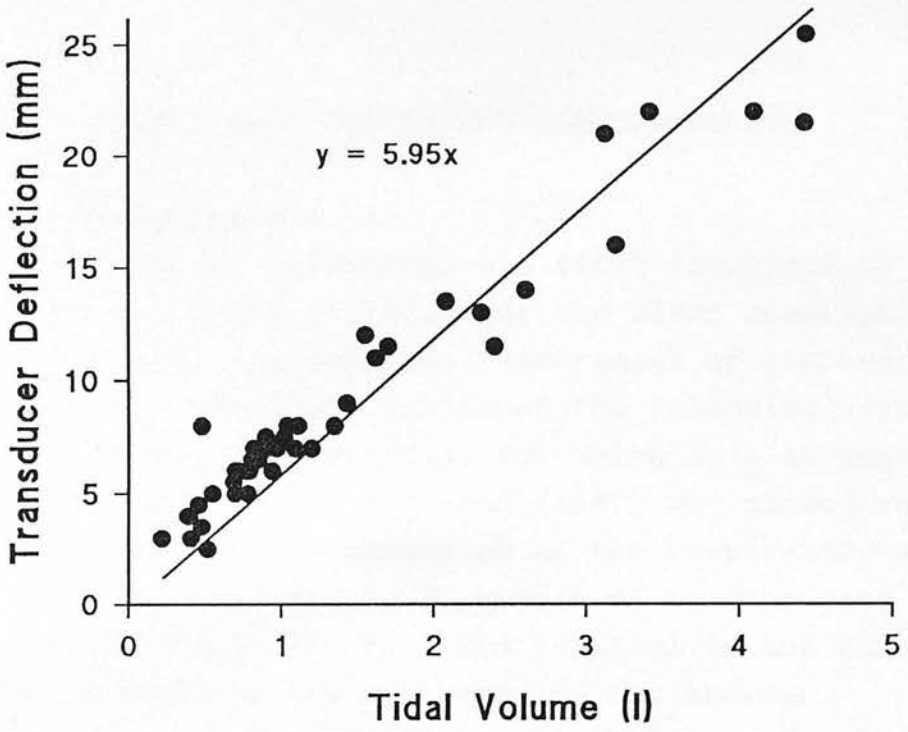

Fig. 2.1 - Lectromed respiration transducer: example of a calibration curve.

volumes as calculated from the transducer output using the calibration curves were within ± 10% of the values measured simultaneously with a spirometer (n = 11). A mean of 57 (SD: 22) % of calculated breath volumes were within ± 20% of spirometer values (n = 11).

The main limitation of the technique is that it assumes that the thorax moves with a single degree of freedom during respiration. As discussed below a more reliable estimate of the volume changes of the lungs is obtained when account is taken of the diaphragmatic contribution by separate measurement of abdominal wall motion. Therefore a technique employing such measurement was used for subsequent studies when it became available to me.

Despite the limitations of the technique it proved adequate to answer the main questions posed in the

studies in which it was used, as described in Chapters 3 and 4.

2.2.3 - RESPIRATORY INDUCTANCE PLETHYSMOGRAPH

a) - Description and Use

An inductive plethysmograph was first described by Milledge and Stott in 1977, but the first description of a device employing separate measurement of rib cage and abdominal movement was published the following year (Cohn et al., 1978). The rationale for using such an approach was provided by Konno and Mead (1967) who showed that during breathing the behaviour of the respiratory system could be approximated by 2 degrees of freedom such that the volume change of the lungs is equal to the sum of the volume changes of the rib cage and the abdomen.

The respiratory inductance plethysmograph (RIP; Respitrace Corporation, Ardsley, New York, USA) consists of 2 coils of Teflon insulated wire sewn in a zig-zag fashion onto separate elasticated bands. One band is placed around the rib cage with the upper edge just below the axillae and the other around the abdomen midway between the lower ribs and the iliac crests (Fig. 2.2). The coils are connected to a small oscillator unit. Respiratory movements cause changes in the cross-sectional area of each coil altering its self-inductance and the frequency of the oscillator to which it is connected. The relationship between change in area and frequency is linear (Cohn et al., 1982). The oscillator unit is connected via an optocoupler, to ensure electrical isolation, to a demodulator/calibrator unit. The demodulator provides DC output signals. There is an optional low pass filter with a time constant of approximately 100 s which can be used, in AC coupled mode, to restore signals to a fixed baseline.

For the longer studies described in Chapters 5 and 7 in which subjects were semi-recumbent I used an elasticated net around each subject's torso supplemented

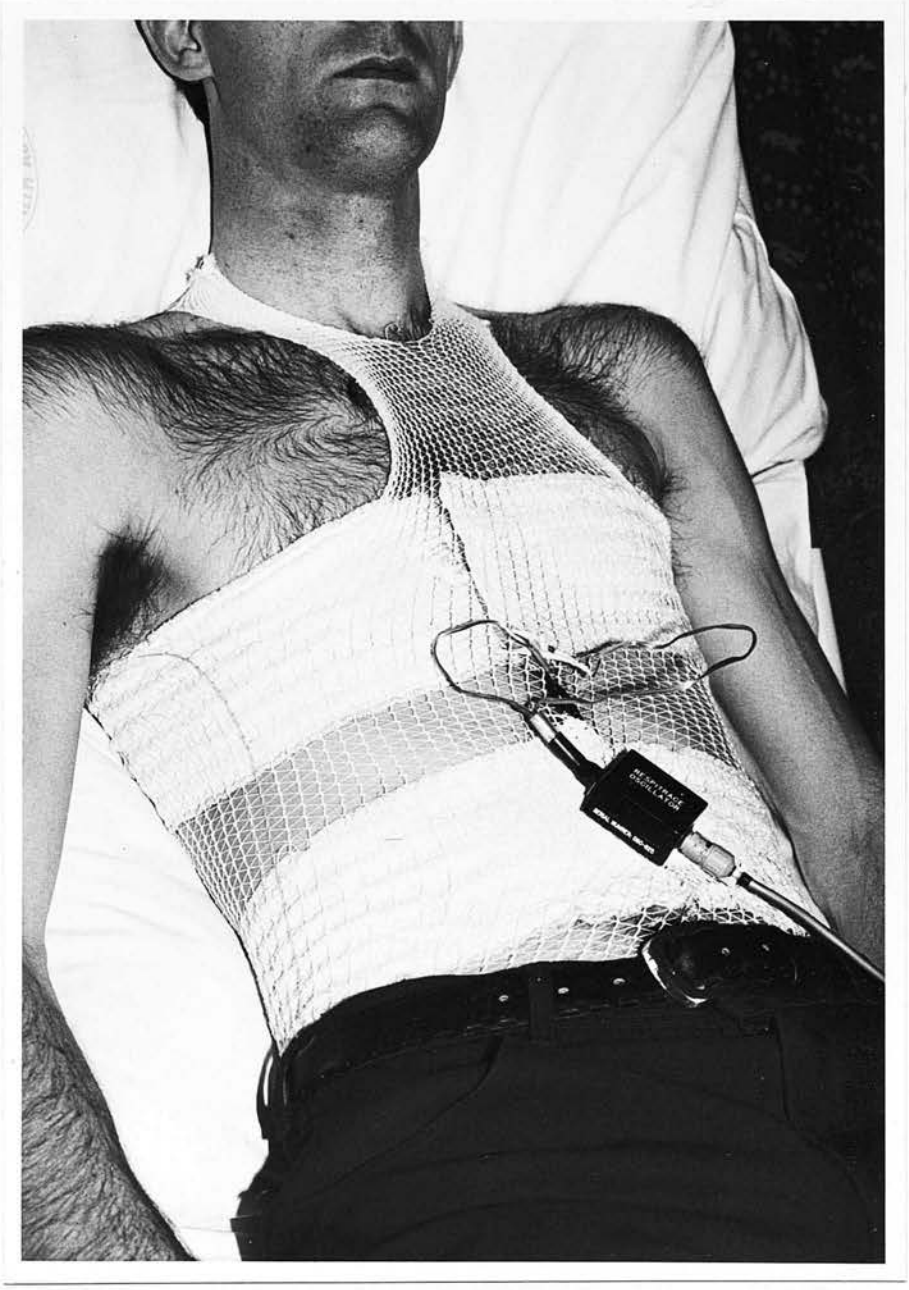

Fig. 2.2 - The respiratory inductance plethysmograph. The rib-cage and abdominal bands can be seen held in place by an elasticated net. The bands are connected to the oscillator which has an output lead to the demodulator/ calibrator unit.

as necessary by sticking plaster to prevent movement of the RIP bands. Positioning marks were also made on each subject's skin so that slippage of the bands could be detected and corrected. These methods were not considered necessary for the shorter studies described in Chapters 6 and 8 in which subjects were recumbent. Where subjects were semi-recumbent a foot board was used to prevent them slipping down the bed since this would have altered their posture and therefore the relative volume-motion coefficients of the rib cage and abdomen.

Prior to all studies the demodulator/calibrator unit was allowed to warm up for at least 1 hour. The unit was always used in DC coupled mode to allow assessment of changes in end-expiratory volume from deviations of the baseline. The RIP signals and, when necessary, the spirometer signal were recorded on a chart recorder (Rikadenki, Model R-03). In 3 studies (Chapters 5, 6 and 8) respiratory frequency and tidal volume were determined manually and used to calculate minute ventilation. For the study described in Chapter 7 the RIP signals were fed to both the chart recorder and to a BBC Master microcomputer, via its analogue to digital converters. A computer program (Respitrace Management Programme kindly supplied by Dr. J. R. Stradling, Radcliffe Infirmary, Oxford) was used to generate a breath-by-breath analysis of respiratory variables and to determine mean values during measurement periods. I found that the frequency of respiration, tidal volume and minute ventilation determined using this program correlate very highly with values obtained manually from a simultaneous chart recording. Artefacts, eg due to coughing or talking, were demonstrated by inspection of the traces recorded and were excluded from the analyses.

b) - Calibration

Based on the observations of Konno and Mead (1967) volume change of the lungs (δVOL) can be expressed as:

$$\delta VOL = A.\delta RC + B.\delta ABD \qquad \text{(Eq. 2.1)}$$

where δRC and δABD represent the change in the RIP signals of the rib-cage and abdomen respectively and A and B represent volume-motion coefficients linking change in these signals to change in volume. Once determined A and B can be used as gain factors for the corresponding RIP signals. The change in the sum of these signals, which can also be obtained from the demodulator/ calibrator unit, then becomes proportional to the change in lung volume.

A number of techniques have been described for the determination of A and B in Eq. 2.1. Two were used in this study and are described here and discussed further in section c) below.

1) Change in posture technique

Based on the method of Chadha et al. (1982) this relies on the variation in relative contributions of rib cage and abdominal movement in different postures. Abdominal and rib cage excursions predominate in the supine and standing positions respectively (Sharp et al., 1975). Subjects recorded at least 10 breaths in both standing and supine positions whilst breathing into a rolling-seal spirometer (Ohio 840). Abdominal and rib cage RIP signals, with gains of both set to 1, and the spirometer signal were recorded simultaneously. Peak heights of the signals were measured and the graph of δRC/δV vs. δABD/δV plotted. This gives 2 clusters of points corresponding to the 2 postures. A regression line was then fitted using the method of least squares (Fig. 2.3).
Rearrangement of Eq. 2.1 gives:

$$\frac{\delta RC}{\delta V} = \frac{1}{A} - \frac{B.\delta ABD}{A.\delta V} \qquad \text{(Eq. 2.2)}$$

From this it can be seen that the X and Y-axis intercepts of the graph represent 1/B and 1/A respectively.

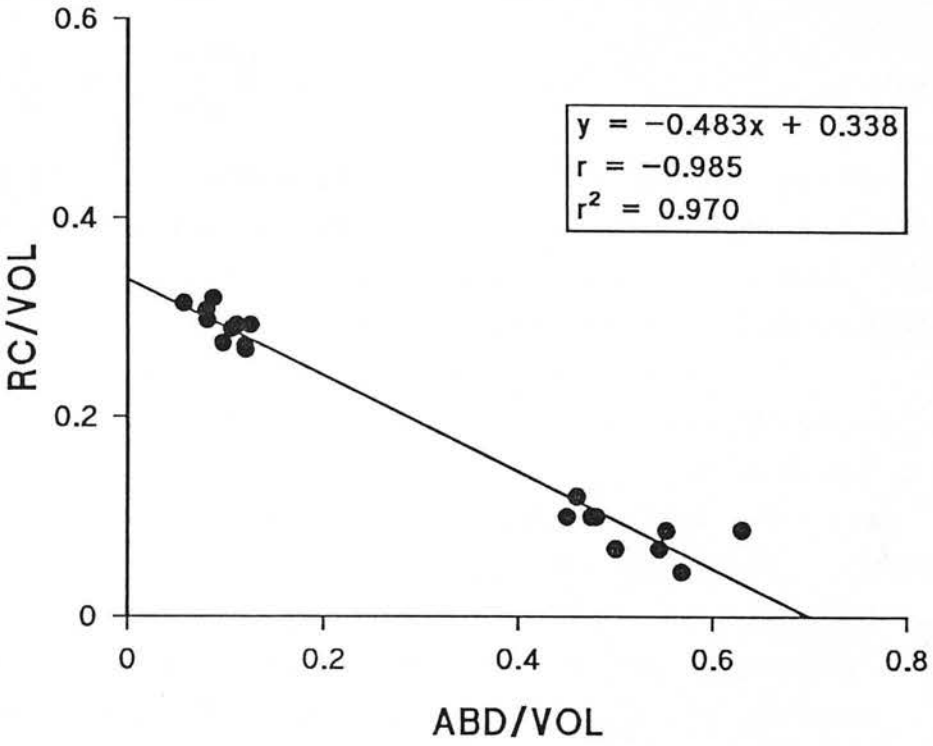

Fig. 2.3 - Respiratory inductance plethysmograph: example of data obtained by changing posture from lying to standing and regression line used to determine rib-cage and abdominal volume-motion coefficients as described in the text. RC, ribcage deflection; ABD, abdominal deflection; VOL, corresponding spirometer deflection.

2) Isovolume technique

Konno and Mead (1967) also showed that during isovolume manoeuvres, in which, with the mouth piece occluded, a subject shifts air from the thorax to the abdomen by voluntary movements of his abdominal wall, the movements of the rib cage and abdomen show a high degree of dependency. The movements are paradoxical and since:

$$A.\delta RC = -B.\delta ABD \qquad \text{(Eq. 2.3)}$$

it follows that:

$$\frac{A}{B} = -\frac{\delta ABD}{\delta RC} \qquad \text{(Eq. 2.4)}$$

Subjects therefore performed isovolume manoeuvres in the posture maintained during the study and the mean of at least 10 estimations of $-\delta ABD/\delta RC$ was obtained. This estimate of A/B was used to set the rib cage signal gain with the abdominal signal gain set to 1.

In the first study in this thesis in which the RIP was used (Chapter 5) the 2-posture technique was used. In all later studies (Chapters 6 to 8) the isovolume technique was used because of its simplicity. After determination of the absolute or relative volume coefficients by either method a final comparison was made between the RIP and a spirometer, in the posture maintained during the study, to obtain accurate volume calibration (Fig.2.4). A rolling-seal spirometer (Ohio 840) was used in Chapters 5 and 8 and a Pocket Spirometer in Chapters 6 and 7. All volumes are expressed at body temperature, ambient pressure and saturation with water vapour (BTPS).

c) - Reliability

Methods used for the calibration of RIP broadly fall into 2 groups: those that employ a change in body posture to obtain breaths of varying abdominal and ribcage contribution and those that perform calibration in a single posture.

Cohn et al. (1982) described a 2-posture technique in which 2 simultaneous equations of the form of Eq. 2.1 were solved to obtain values for A and B. More recently a least squares regression technique similar in concept to that described above was developed (Chadha et al., 1982). These methods have been criticised on the ground that a change in posture induces changes in the relative volume-motion coefficients of the abdomen and rib cage. For instance Zimmerman et al. (1983) found that a change

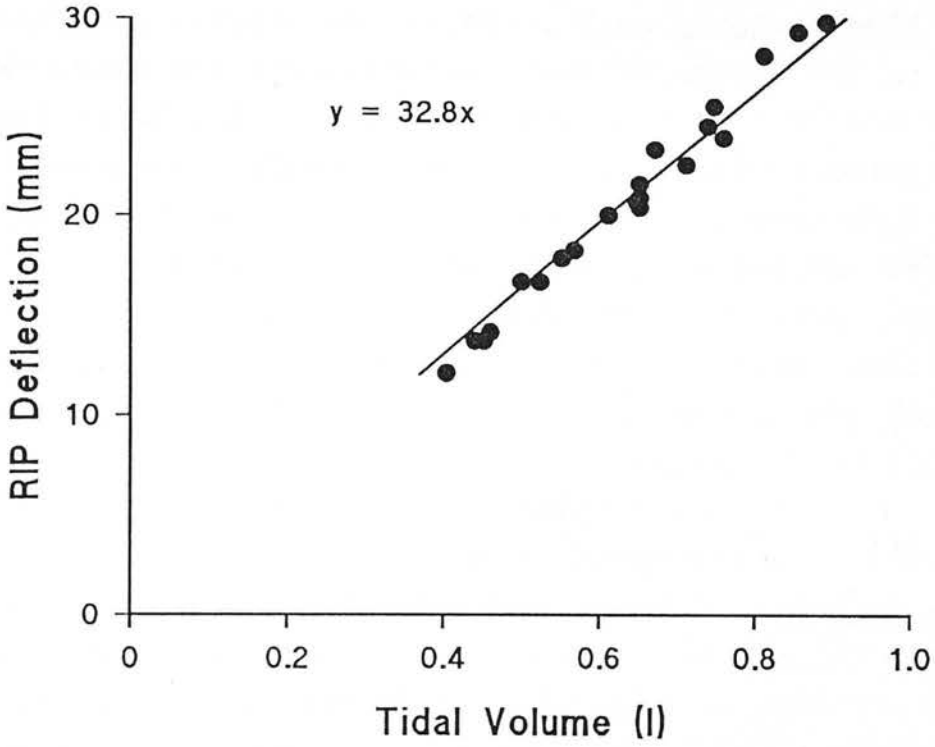

Fig.2.4 - Example of a volume calibration curve for the respiratory inductance plethysmograph, performed after initial determination of the rib-cage and abdominal volume-motion coefficients using the two posture technique.

from the standing to the semirecumbent position caused changes of +20% and -21% in the rib cage and abdomen coefficients respectively. Using a 2-posture calibration technique these authors found that measurements of tidal volume by RIP deviated a mean of 16 to 17% from spirometer values in different postures. However they did not recalibrate RIP for absolute volume in each posture used for comparison. They also acknowledged that a tight linear relationship can exist between RIP and spirometer estimations of tidal volume in any position provided that the relative contributions of the rib cage and abdomen are nearly constant over the range of breaths measured.

Despite the theoretical criticisms of the 2-posture

techniques several workers have found them to give acceptable results in practice. Cohn et al. (1982) found that using the simultaneous equation method 86% of mean tidal volumes in 7 different postures fell within ± 10% of spirometer values. Chadha et al. (1982) similarly found 93% of mean tidal volumes in 2 postures fell within ± 10% when they used a least squares technique. In their hands this was more precise than they achieved using an isovolume technique, where 83% in the supine position and 90% in the standing position fell within ± 10%. However others have found the 2 techniques equally good (Gonzalez et al., 1984; Dadzie et al., 1985).

The most widely used single posture calibration technique has employed the isovolume manoeuvre as described above. This has been criticised because some subjects find the manoeuvre difficult to perform. An interaction between the rib cage and abdomen during the manoeuvre may lead to a consistent underestimation of the abdominal volume motion coefficient (Stradling et al., 1985). However Stradling et al. (1985) found the isovolume technique only slightly less reliable in normal subjects than a more sophisticated multiple linear regression technique. The latter technique relied on phase shifts between the abdomen and rib cage during ordinary breathing and multiple sampling of points within each breath to generate sufficient scatter in rib cage and abdomen contributions to allow computation of the volume motion coefficients. In patients with chronic obstructive airways disease the 2 calibration techniques were equally good (Stradling et al., 1985).

Several workers have examined the reliability of the RIP in patients with chronic respiratory disease. It is recognised that a significant proportion of such patients may manifest abnormalities of thoracoabdominal motion such as indrawing of the costal margins on inspiration or paradoxical motion of the rib cage and abdomen (Ashutosh et al., 1975; Sharp et al., 1977). Nevertheless several studies have shown that with care the RIP can be used in

such patients with an acceptable degree of accuracy (Tobin et al., 1983; Gonzalez et al., 1984; Dadzie et al., 1985; Stradling et al., 1985). In these patients the estimates of the proportion of mean tidal volumes measured by RIP lying within ± 10% of spirometer values range from 57% (Dadzie et al. (1985) to 100% (Tobin et al., 1983; Gonzalez et al., 1984) with 1- or 2-posture calibration techniques equally reliable.

In view of the previous reports on the reliability of the RIP a formal validation was not performed as part of this work. However one indication of the precision of the technique is provided by the scatter of values about the regression lines obtained during volume calibration. In the study in normal subjects described in Chapter 5 a mean of 91 (SD: 10) % of breath volumes as calculated from the RIP output using the calibration curves were within ± 10% of the values measured simultaneously with a spirometer (n = 10). Furthermore a mean of 61 (SD: 17) % were within ± 5% of spirometer values (n = 10).

A criticism of my use of the RIP is that the calibration was not checked after any study except the one described in Chapter 7. However all studies except those described in Chapters 5 and 7 were short, which would make the occurrence of significant changes in calibration less likely. It is conceivable that such changes occurred in the longer study described in Chapter 5. However during the "placebo leg" of that study (see Chapter 5) mean baseline minute ventilation before the final infusion stage differed by only 5% from the value before the first adenosine infusion stage. It is therefore unlikely that a significant systematic change occurred.

In the study in patients with chronic obstructive airways disease a check calibration was performed after the study and revealed a change in the volume calibration slope in some patients. This is discussed further in Chapter 7. Had changes in calibration of a similar magnitude occurred in any of the other studies the

possible resultant errors in measurement of minute ventilation would have been small compared with the changes caused by adenosine. It is therefore unlikely that any such change would have affected the conclusions.

2.2.4. - DISCUSSION

It has long been recognised that conscious and psychological factors can act as stimuli to breathing (Krogh & Lindhard, 1913). Even knowledge by a subject that he is undergoing an intervention that can affect respiration can do this (Barcroft et al., 1957). As far as possible such factors were avoided, eg by keeping unnecessary noise to a minimum. It was considered unethical not to inform subjects of the expected effects of adenosine. No patient had received adenosine previously but some of the normal subjects in the study described in Chapter 3 had. At the end of each infusion stage each subject was asked to report any symptoms and asked if he/she was willing to continue to the next stage. For this reason subjects were aware when infusions were being given. However all studies except those described in Chapters 4 and 6 employed a placebo or comparison infusion, with all infusions being given single-blind. Where subjects were studied on more than one occasion (Chapter 5) studies were done at the same time of day.

2.3 - MEASUREMENT OF ADENOSINE CONCENTRATIONS

2.3.1 - INTRODUCTION

Several methods have been used to determine concentrations of nucleosides, such as adenosine, in biological materials. Previously one of the most widely used was a modification (Olsson, 1970) of a spectrophotometric method first developed by Kalckar (1947a,b). This method is, however, insensitive and requires large sample volumes. Numerous other methods have therefore been developed, including radioenzymatic

(Namm & Leader, 1974), competitive protein binding (Olsson et al., 1978), enzymatic fluorometric (Gardiner, 1979; Slowiaczek & Tattersall, 1982), radioimmunoassay (Schrader et al., 1978; Sato et al., 1982), HPLC (Hartwick & Brown, 1977; Pfadenhauer & Tong, 1979; Fredholm & Sollevi, 1980) and chemiluminescent (Kather et al., 1987).

Techniques using high performance liquid chromatography (HPLC) have recently been the most widely used, but some earlier methods lacked sensitivity (Hartwick & Brown, 1977). One refinement which provides greater sensitivity than methods using detection by ultraviolet absorption (Jacobson et al., 1983), has been the use of fluorometry to detect adenosine after its conversion to a fluorescent derivative (Barrio et al., 1972; Kuttesch et al., 1978). Such a method was used in the present work. This was developed by WJ Penny and AC Newby (personal communication) as a modification of the method of Wojcik and Neff (1982) in which adenosine is converted to 1,N^6-ethenoadenosine prior to quantification. This method is described below.

Several workers have measured blood adenosine concentrations in man using various techniques. Reported values show considerable variation, for reasons that are not always immediately apparent. In peripheral venous plasma from normal volunteers the adenosine concentration has been found to range from undetectable (Hartwick & Brown, 1977) to 2 μM (Slowiaczek & Tattersall, 1982). Examples illustrating the range of reported values and techniques used are shown in Table 2.1.

Several authors have stressed the importance of the blood sampling technique. As discussed in Chapter 1 red blood cells possess avid uptake mechanisms for adenosine which then undergoes intracellular metabolism. Various workers have shown that when small amounts of radiolabelled adenosine are added to human blood, the activity in plasma rapidly falls with a half-life of < 10s (Tattersall et al., 1983; Klabunde, 1983; Ontyd &

Table 2.1 - Some previous reports of mean (SD) basal plasma adenosine concentration in man.

Reference	Sampling method	Assay procedure	[adenosine] (μM)	n
Hartwick & Brown (1977)	1 ml acid precipitation	reversed phase HPLC ultraviolet (UV) detection	undetectable	
Kuttesch et al. (1978)	added to heparin acid precipitation	adenosine derivatised to ethenoadenosine reversed phase or cation exchange HPLC fluorometric detection	0.07 (0.03)	6
Pfadenhauer & Tong (1979)	5 ml sample placed in ice bath ultrafiltration	purification with boronate affinity gel reversed phase HPLC UV detection	0.23 (0.18)	6
Capogrossi et al. (1982)	7 ml into heparin & 2′-deoxycoformycin (adenosine deaminase inhibitor) cooled in ice ultrafiltration for 2 h	reversed phase HPLC	plasma: 0.51 (0.14) serum: 5.7 (1.7)	11 4
Slowiaczek & Tattersall (1982)	into cold heparin & EHNA ultrafiltration	purification with boronate affinity gel enzymatic fluorometric	2.0	5
German et al. (1984)	into 2′-deoxycoformycin and heparin acid precipitation	radioenzymatic HPLC to separate synthesized AMP	males: 0.121 (0.054) females: 0.101 (0.067)	 21 16
Ontyd & Schrader (1984)	2 ml into ice-cold solution of dipyridamole etc. using double-syringe technique acid precipitation	reversed phase HPLC repeated after enzymatic peak shift UV detection	0.29 (0.13)	5
Hamm et al. (1988)	0.5 ml into ice-cold saline acid precipitation	radioimmunoassay	arterial: controls: 0.067 (0.037) patients (CAD): 0.095 (0.061)	 3 7
Möser et al. (1989)	into solution of EHNA and dipyridamole using dual-chambered syringe acid precipitation	radioimmunoassay	0.072 (0.008)	6

Schrader, 1984). Adenosine transport into cells is inhibited by various compounds, of which the most studied is dipyridamole. At 10^{-6} to 10^{-5} M dipyridamole causes almost complete inhibition of adenosine uptake into red cells (Koss et al., 1962; Bunag et al., 1964; Klabunde, 1983). Several workers have therefore mixed blood with dipyridamole solution either during or following sampling (Ontyd & Schrader, 1984; Grum et al., 1985; Biagionni et al., 1986). Ontyd and Schrader (1984) devised a double-syringe technique to allow rapid mixing of blood with a "stopping" solution containing dipyridamole during blood sampling (Fig. 2.5). Slowiaczek and Tattersall (1982) however found that when blood was sampled into a solution containing erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA), an inhibitor of adenosine deaminase, (discussed further below) the addition of dipyridamole made little difference to the measured adenosine concentration. These workers only however measured basal adenosine concentrations. Cellular uptake of adenosine may have more effect on plasma adenosine concentrations when the latter are increased by the addition of exogenous adenosine (see Discussion below).

Addition to sampled blood of inhibitors of adenosine deaminase such as EHNA, has been shown to result in higher measured adenosine concentrations (Slowiaczek & Tattersall, 1982; Tattersall et al., 1983). Capogrossi et al. (1982) could only detect adenosine in plasma when another such inhibitor, 2'-Deoxycoformycin, was used.

Blood clotting has been shown to cause marked liberation of adenosine (Capogrossi et al., 1982) possibly from ADP released by activated platelets or nucleotides released from damaged red cells. Adenosine from such sources may have contributed to the high concentrations of adenosine reported by some workers. Anticoagulation of blood following sampling has been employed by several workers, eg Kuttesch et al. (1978), Capogrossi et al. (1982) and Slowiaczek and Tattersall (1982). This may not however be necessary provided plasma

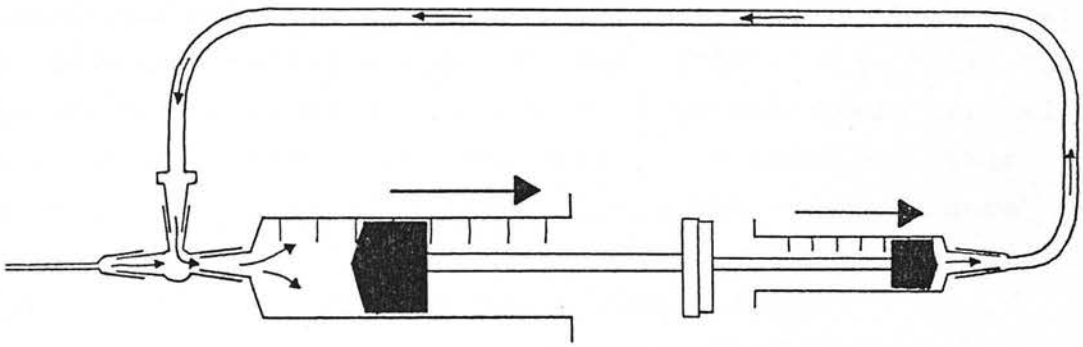

Fig 2.5 - "Double-syringe" used for sampling blood for measurement of adenosine concentration. As blood is drawn into the larger syringe it is mixed with an ice-cold "stopping solution", containing inhibitors of adenosine metabolism, expelled from the smaller syringe.

is quickly separated and deproteinised. Furthermore dipyridamole, if used, inhibits platelet aggregation and thereby ADP liberation during the release reaction (Born & Cross, 1963). Even when they used anticoagulation Slowiaczek and Tattersall (1982) found that plasma purines gradually increased. This was partially but not completely prevented by cooling to 4°C. Following removal of cellular elements plasma purines were "relatively stable" provided enzyme inhibitors were present.

The blood sampling technique used in the present work employed rapid mixing of sampled blood with a "stopping" solution, rapid cooling and rapid separation and deproteinisation of plasma.

2.3.2 - METHOD USED IN THIS STUDY

a) - Blood sampling

Blood (approximately 3 ml) for measurement of adenosine concentration was mixed during sampling with 1.5 ml of an

ice-cold "stopping" solution to inhibit metabolism of adenosine. For the study described in Chapter 5 the mixing was achieved by use of a double-syringe technique, as described by Ontyd and Schrader (1984) (Fig. 2.5). For the study described in Chapter 6 blood was drawn directly into syringes containing the "stopping" solution. This solution consisted of dilazep (Astra-Werke AG), a more potent inhibitor of cellular transport of adenosine than dipyridamole and EHNA (final concentrations: approximately 130 μM and 10 μM respectively) in 0.9% sodium chloride.

b) - Extraction

A 3 ml aliquot of the diluted sample was immediately separated by centrifugation (Eppendorf Model 5413) at 8000 g for 30 s and 1.2 ml of the supernatant was deproteinised by addition of an equal volume of 10% (w/v) trichloroacetic acid at 4°C. The mixture was allowed to stand on ice for at least 5 min and was then centrifuged at 1200 g for 15 min at 4°C. The deproteinised supernatant was neutralised using paired ion extraction (Khym, 1975) by shaking it with a mixture of tri-n-octylamine (0.9 g) and 1,1,2-trichlorotrifluoro-ethane (5 ml) until the pH was at least 5.5. The aqueous phase was filtered (Millipore GS 0.22 μm) and then stored at -20°C for later analysis.

c) - Assay

Samples (0.5 ml) of neutralised deproteinised plasma were incubated with 0.05 ml of 0.5 M ammonium acetate buffer (pH 5) and 0.025 ml of 4 M bromoacetaldehyde for 20 min at 80°C to convert adenosine to 1,N6-ethenoadenosine. The reaction was stopped on ice. Aqueous solutions of standard concentrations of adenosine (0, 0.0025, 0.025, 0.25 and, in some cases, 1 μM) were also processed in this way. The resultant ethenoadenosine standards were used to construct a 4-point calibration curve for each batch of samples assayed (Fig. 2.6). There was a linear

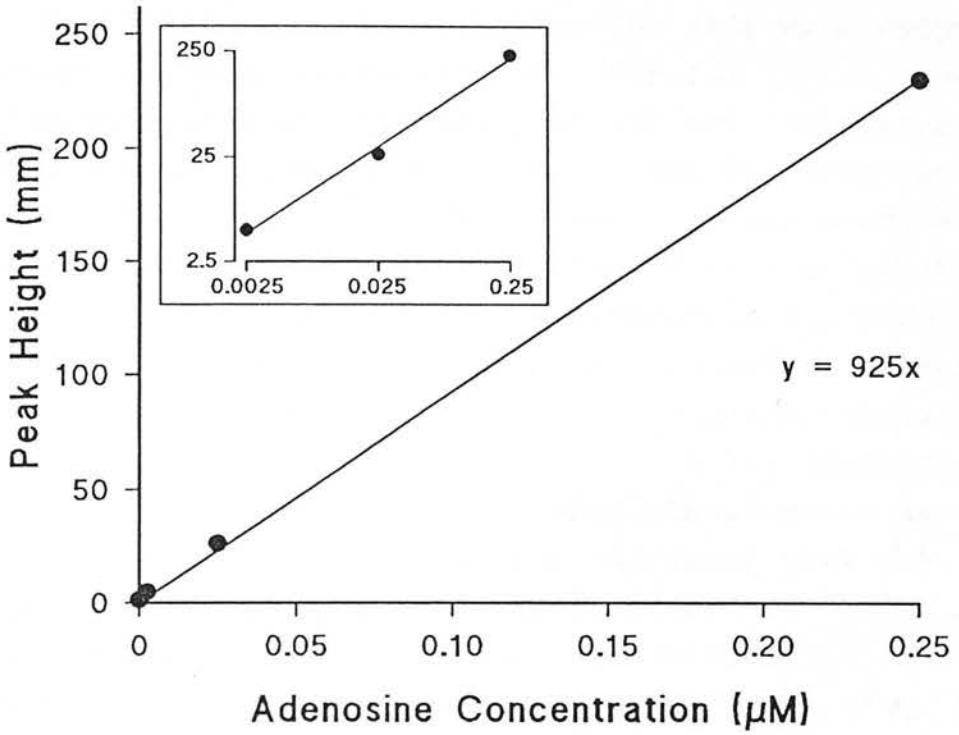

Fig. 2.6 - Adenosine assay: typical calibration curve. The inset shows the data (excluding the point for a concentration of 0 μM) plotted on a log-log curve (see section 2.3.3).

relationship between the ethenoadenosine fluorescence peak heights and the adenosine concentrations ($r > 0.999$). Peak heights were therefore used for measurement of samples.

Penny and Newby (personal communication) showed that conversion to ethenoadenosine of standard adenosine (0.25 μM) added to deproteinised plasma was 92 (SD: 4) % (n = 10) after taking into account the endogenous adenosine in plasma. I compared chromatographic peak heights of aqueous solutions of commercially produced etheno-adenosine and derivatised standard adenosine (both 0.025 μM). This suggested conversion of 102 (SD: 18) % (n = 6) of adenosine to ethenoadenosine.

1,N6-ethenoadenosine was quantified by HPLC using

fluorometric detection (excitation at 254 nm and emission at 300 to 400 nm) (LDS Fluoromonitor lll) with output to a chart recorder (Servogar BBC). Samples (200 μl) were chromatographed isocratically at ambient temperature on a 250 x 4.6 mm column of Apex ODS (Jones Chromatography Ltd., Llanbradach, S. Glam.) using ammonium acetate buffer (pH 4.5) containing 7% acetonitrile as eluate at a flow rate of 1.5 ml/min (LDC Constametric lll pump).

Ethenoadenosine in samples was identified primarily by retention time, determined from injected standard solutions of ethenoadenosine. For selected samples a duplicate was either "spiked" with ethenoadenosine to increase the peak height or the adenosine peak was removed by incubating the sample, prior to derivatisation, with adenosine deaminase which converts adenosine to inosine (Wojcik and Neff, 1982) (Fig. 1.2). Addition of 10 μl of an aqueous solution containing 0.9 units (0.016 mg) of adenosine deaminase per 200 μl aliquot of the neutralised deproteinised plasma and incubation at room temperature for 30 min resulted in almost complete removal of the adenosine peaks in the samples tested (Fig. 2.7).

d) - Quantification

The plasma concentration of adenosine was determined from the measured concentration of ethenoadenosine by the following equation:

$$[\text{adenosine}] = [\text{ethenoadenosine}] \times 2 / A \qquad \text{(Eq. 2.5)}$$

where the factor 2 corrects for dilution by trichloroacetic solution and A corrects for dilution by the stopping solution during sampling:

$$A = \frac{(1 / H_1) - 1}{(1 / H_2) - 1} \times \frac{M_1}{M_2} \qquad \text{(Eq. 2.6)}$$

Here H_1 and H_2 are the haematocrits of the patients blood before and after dilution by the stopping solution, and M_1 and M_2 are the red cell mean corpuscular volumes

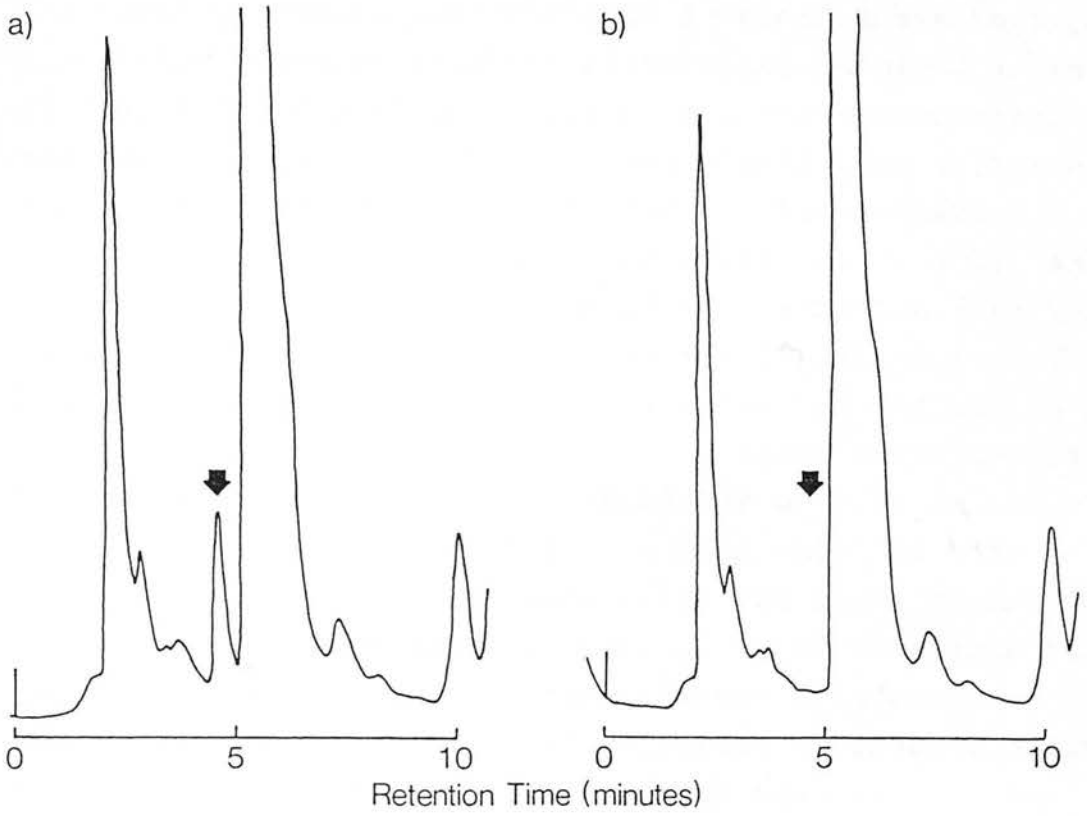

Fig. 2.7 - Adenosine assay: a) typical chromatogram showing ethenoadenosine peak (arrow); b) a duplicate sample showing removal of the ethenoadenosine peak by prior incubation of the sample with adenosine deaminase.

before and after dilution. The correction for red cell size was made because I found that M_2 was often slightly larger than M_1, possibly because of an osmotic effect of the stopping solution.

e) - Reliability of the blood sampling technique

Sampling of blood through a catheter as was done in the study described in Chapter 6 poses particularly problems. Firstly adenosine might be liberated into plasma from adenine nucleotides released from activated platelets or damaged red cells. Secondly the very rapid turnover of adenosine in blood might reduce the plasma concentration.

Penny and Newby (personal communication) found that the basal adenosine concentration in blood drawn through a catheter might be slightly higher than in blood drawn through a short cannula. I found in a small comparison in one subject that there was no significant difference between the adenosine concentration in blood drawn through a short cannula, 0.05 (SD: 0.01) μM (n = 2), and blood sampled through a narrow plastic infusion line 1 m long connected to a cannula, 0.06 (SD: 0.01) μM (n = 6). However the 95% confidence interval for the difference is consistent with the concentration in blood drawn through the infusion line being 0.04 μM higher or 0.01 μM lower than blood sampled through the cannula only. If this indicates the maximum expected error for basal adenosine concentrations when sampling through a catheter then this is acceptable for studies where changes in adenosine concentration of the magnitude reported in Chapter 6 (up to 2 μM) are found. The large changes observed during adenosine infusion in that study are unlikely to have been due to blood clotting in the catheters, since the values at baseline and following the infusion were the same (see Chapter 6).

When plasma adenosine concentration is acutely increased net transport into cells occurs. Möser et al. (1989) showed that in vitro, at plasma concentrations in the range 1 to 5 μM, exogenous adenosine has a half-life of approximately 1.5 s. The transit time of blood through the sampling catheters in the study described in Chapter 6 was approximately 0.75 s, as estimated in vitro. Therefore the measured concentrations, at the highest infusion rates of adenosine, may have underestimated, but were very probably of the same order of magnitude as, the true values.

Comparison of the double syringe technique with a single syringe technique showed no significant difference in mean plasma concentrations obtained. With the single syringe the measured concentration in a single subject was 0.06 (SD: 0.02) μM (n = 4) compared with 0.05 (SD:

0.01) μM (n = 4) with the double syringe technique. The difference between the variances was not significant but the 95% confidence interval was compatible with the variance using the single syringe technique being up to 8-fold higher, i.e. implying a greater scatter of results. Since I found no evidence of a large difference in mean concentrations measured by the 2 sampling techniques the single syringe technique was used for blood sampling down catheters (see Chapter 6) because it was practically much simpler.

f) - Reliability of the assay

Penny and Newby (personal communication) assessed recovery of adenosine during the sampling procedure by adding 2,5,8-^{3}H-adenosine (0.25 μM) to the stopping solution. Chromatography of the neutralised plasma extracts showed recovery of 105 (SD: 4) % (n = 7) of the ^{3}H-adenosine in the adenosine peak. I assessed recovery using a somewhat cruder technique. In a single subject multiple blood samples for measurement of adenosine concentration were taken on two occasions. The stopping solution used for some of these was "spiked" with adenosine at one of three concentrations (0.06 μM, 0.6 μM or 2.4 μM). Recovery of the added adenosine was estimated by comparing the measured adenosine concentration in each "spiked" sample with a paired "unspiked" sample. Estimated recovery at the three concentrations was 108 (SD: 158) %, 107 (SD: 19) % and 113 (SD: 16) % respectively (n = 6 for each estimate).

In my hands the detection limit of the assay was 0.001 μM (equivalent to undiluted plasma adenosine concentration $\leq$ 0.01 μM).

I determined coefficients of variation by assaying paired samples of 2 standard adenosine solutions (0.01 and 1.0 μM). One sample of each pair was assayed at the beginning and one at the end of a batch of assays. Coefficients of variation, C, were calculated using the formula:

$$C = s / \mu \times 100 \qquad \text{(Eq. 2.7)}$$

where s is the standard deviation and μ the mean of a group of measured concentrations. For intra-assay coefficients the mean of values obtained from several paired samples was calculated. Results were:

1) Mean intra-assay coefficients of variation:

9% (n = 8) at 1.0 μM (equivalent to plasma adenosine concentration approximately 5 to 10 μM) and 18% (n = 6) at 0.01 μM (equivalent to plasma adenosine concentration approximately 0.05 to 0.1 μM).

2) Interassay coefficients of variation:

16% (n = 10) at 1.0 μM and 27% (n = 14) at 0.01 μM.

2.3.3 - DISCUSSION

Recently further workers have made pertinent observations on measurement of adenosine concentrations in man. Hamm et al. (1988) reported that neither breakdown of adenosine by plasma enzymes nor binding of adenosine to plasma proteins precipitated during acid extraction caused significant loss of recovery of adenosine. The first of these observations is at variance with some previous reports which are discussed above. Hamm et al. also observed that adenosine was rapidly generated in incubated plasma, presumably from adenine nucleotides, suggesting that enzyme inhibitors should be used to prevent this. However Ontyd and Schrader (1984) previously found that addition of α,β-methylene adenosine 5'-phosphate, an inhibitor of the 5'-nucleotidase responsible for extracellular nucleotide degradation, made no difference to their measured adenosine concentrations. Ontyd and Schrader used a system of blood sampling very similar to that employed in the present work. Hamm et al. (1988) also confirmed the previously reported rapid turnover of adenosine and found that rapid cooling of blood was more effective than dipyridamole in

preventing this.

Möser et al. (1989) recently reported that incubation of heparinised blood for up to 1 hour after sampling had little effect on the basal adenosine concentration suggesting that at such concentrations an equilibrium exists between influx and efflux of plasma adenosine. Furthermore use of a "stopping" solution containing dipyridamole and EHNA during blood sampling had little effect on measured basal values. They also found that degradation of extracellular nucleotides contributed greatly to the formation of adenosine in that steady state. They confirmed the rapid turnover of adenosine and showed that when the plasma adenosine concentration was increased to approximately 1 μM the subsequent fall was rapid with a half-life of 1.4 s. This suggests that measures to inhibit adenosine metabolism during blood sampling are more important when concentrations are increased than at basal values. The implications of the findings of Möser et al. will be discussed further in Chapter 6.

There are several possible criticisms of the present assay. Samples required considerable processing prior to chromatography which was a fairly slow process so that 10 to 15 samples at most could be analysed per day. It became apparent towards the end of the work that large changes in ambient temperature affected the etheno-adenosine peak retention time and the separation of this peak from adjacent peaks. Therefore determination and maintenance of the optimum column temperature might have increased the precision of the assay. Minor changes in the acetonitrile concentration were sometimes necessary to improve separation of the ethenoadenosine peak. For this reason samples were injected manually rather than automatically. An initial purification step, e.g. using phenyl boronate affinity gel as employed by several workers, might have improved the chromatographic separation.

An internal standard was not used. However recovery

during the sampling procedure was high and adenosine standard solutions used for calibration were submitted to the derivatisation process at the same time as samples to correct for incomplete derivatisation to ethenoadenosine. The concentrations of adenosine used for calibration curves were such that the slope of the linear regression line was heavily influenced by the point at the highest concentration (Fig. 2.6). It might have been preferable to calculate calibration curves after plotting the data on a log-log plot. However, as shown in Fig. 2.6 (inset), concentrations determined from such a curve are unlikely to have been very different from those obtained from the non-logarithmic plot. Coefficients of variation were high but, as shown in Chapter 5, the assay was sensitive enough to detect a change in the mean adenosine concentration as small as 0.03 μM.

2.4 - INFUSION TECHNIQUE

All infusions were administered using a Harvard infusion pump, model 2681 and 50 ml plastic syringes. Except where indicated, infusions were given via a plastic cannula inserted in an antecubital vein. Flow rates of 27 to 281 ml/h were used. Successive increments using this pump follow an approximate geometric progression with a ratio of 1.4:1.

Infusion fluids were sterile solutions of adenosine or inosine (Sigma Chemical Company) at a concentration of 5 mg/ml in 0.9% sodium chloride, 0.9% sodium chloride, or aminophylline (6 mg/kg in 30 ml sterile water). Solutions of adenosine and inosine for human use were prepared according to usual quality control criteria by the Pharmacy Department, University Hospital of Wales, Heath Park, Cardiff.

2.5 - GENERAL CONSIDERATIONS

Except where indicated subjects were asked to abstain from caffeine-containing beverages on any day of study.

2.6 - ETHICAL AND SAFETY CONSIDERATIONS

Protocols for all studies were submitted to and approved by the ethics committee of the Division of Medicine, South Glamorgan Health Authority. All subjects gave informed, written consent to their inclusion in the relevant study.

With one exception (see Chapter 5) patients or volunteers with a history of asthma were not studied, because of the known bronchoconstrictor effect of inhaled adenosine in such patients. Aminophylline, which was considered to be a probable antagonist of the cardiovascular and respiratory effects of adenosine, was available for intravenous administration if necessary during all studies. All subjects were aware that any infusion would be stopped at any point at their request.

2.6 - STATISTICAL METHODS

Standard statistical methods were used throughout (Zar, 1984). Individual methods used are indicated in each chapter. A frequently used method was repeated measures (randomised block) analysis of variance (referred to in this thesis as ANOVA). For occasional missing values estimates were obtained and the degrees of freedom for ANOVA were appropriately modified using the method of Glenn and Kramer (1958). Computation was performed using an IBM compatible personal computer (Amstrad 1640, Amstrad PLC) and a spreadsheet (Ability, Migent (UK) Ltd.) or a pocket personal computer (Casio FX-720P, Casio Computer Company Ltd., Japan). Some of the programmes for the latter were written by Professor Philip Routledge. All other programmes were written by the author. In all analyses significance was assumed if P was less than 0.05, unless otherwise stated.

CHAPTER 3 - A COMPARISON OF THE CARDIOVASCULAR AND RESPIRATORY EFFECTS OF INTRAVENOUS INFUSION OF ADENOSINE AND INOSINE

3.1 - INTRODUCTION

As discussed in Chapter 1 Watt and Routledge (1985) observed dose-dependent stimulation of respiration in man produced by intravenous boluses of adenosine. In view of the very short half-life of adenosine significant metabolism of adenosine would be expected during the 15 to 20 s interval Watt and Routledge observed between injection of adenosine and the onset of respiratory stimulation. It was therefore unclear whether the observed effects on respiration were due to adenosine itself or a metabolite.

Furthermore adenosine had been reported to cause hypotension when infused during anaesthesia (Sollevi et al., 1984a). If a similar effect was produced in conscious subjects this might cause respiratory stimulation (Landgren & Neil, 1951; Heistad et al., 1975). Watt and Routledge did not measure blood pressure during their studies with adenosine boluses.

The adenosine-induced increase in respiration observed by Watt and Routledge was a transient phenomenon following intravenous bolus injections. It was not known whether a sustained effect would be seen during continuous infusion.

To address the above points the effects on respiration, heart rate and blood pressure of intravenous infusions of adenosine and its metabolite inosine were compared.

3.2 - SUBJECTS AND METHODS

3.2.1 - SUBJECTS

The subjects were 8 healthy volunteers (7 male) aged 23 to 33 years (Appendix 1).

3.2.2 - INFUSION PROTOCOL

Adenosine and inosine were administered in random order, single-blind, by intravenous infusions separated by 30 min. This interval was chosen on the basis of the finding in a pilot study that the cardiorespiratory effects produced by adenosine infusion resolve within 1 to 2 min of stopping the infusion. The infusion rate of each nucleoside was initially 3.1 mg/min and was increased stepwise every 2 min up to a possible maximum of 23.4 mg/min (maximum possible number of stages: 7). Each infusion was discontinued following the maximum dose or earlier at the request of a subject.

3.2.3 - MEASUREMENTS

A single-lead electrocardiogram (ECG) was monitored throughout each infusion. Recordings of the ECG and measurements of blood pressure (using an Accoson mercury sphygmomanometer, taking phase five as diastolic) were made at baseline and at 1 min intervals throughout each infusion. Respiration was recorded using a respiration transducer (Lectromed type 4320) as described in Chapter 2. End-tidal PCO_2 ($PETCO_2$) was measured continuously in gas sampled by a fine-bore cannula, whose tip was clipped to the upper front teeth, using a high speed response CO_2 analyser (Type 901 MK 2. PK Morgan Limited). The respiratory and PCO_2 traces were recorded on an Ormed MX216 recorder. Respiratory variables were subsequently derived from the traces obtained: respiratory rate (f_R), tidal volume (V_T), minute ventilation (V) and $PETCO_2$ at 1 minute intervals, and inspiratory duration (T_I), expiratory duration (T_E), total breath duration (T_{TOT}) and mean inspiratory flow rate (V_T/T_I) at baseline and at the maximum doses of each compound. The end-expiratory plateau in the CO_2 trace was used since this provides an approximate measure of the arterial PCO_2 (Bülow, 1963). Subjects were asked to report any symptoms at 1 min intervals and were aware that an infusion would be stopped immediately at their request. In four subjects

(nos. 4,5,6 and 7) spirometry was performed prior to and immediately after each infusion using a Pocket Spirometer (Micromedical Instruments, Strood, Kent).

3.2.4 - STATISTICAL ANALYSIS

Comparisons of respiratory rate, tidal volume, minute ventilation, $PETCO_2$, heart rate and blood pressure at different infusion rates, up to 8.5 mg/min for adenosine and 16.8 mg/min for inosine, were made using repeated measures analysis of variance and Student-Newman-Keuls test. Student's paired t-test was used to compare baseline values of the above variables as well as V_T/T_I and inspiratory duration over total breath duration (T_I/T_{TOT}) with values at the maximum dose of each nucleoside, and to compare spirometric variables (peak expiratory flow rate (PEFR), forced expiratory volume in 1 s (FEV_1) and forced vital capacity (FVC)) before and after each infusion. In one subject (no. 8) the study was stopped because of occipital headache and in one subject (no. 7) an inadequate respiratory trace was obtained. All data, apart from symptoms and spirometry, were therefore analysed for six subjects.

3.3 - RESULTS

The maximum dose rates received ranged from 8.5 to 23.4 mg/min (mean: 13.5, SD: 5.7 mg/min) for adenosine and 16.8 to 23.4 mg/min for inosine. Data are therefore presented for infusion rates up to 8.5 mg/min and for the maximum infusion rate received by each subject for adenosine and for infusion rates up to 16.8 mg/min for inosine.

3.3.1 - CHANGES IN RESPIRATION DURING ADENOSINE INFUSION

The effects of adenosine infusion on respiratory rate, tidal volume, minute ventilation and $PETCO_2$ are shown in Fig. 3.1. During adenosine infusion minute ventilation increased significantly from 6.6 (SD: 4.5) l/min at baseline to 13.0 (SD: 5.7) l/min at an infusion rate of

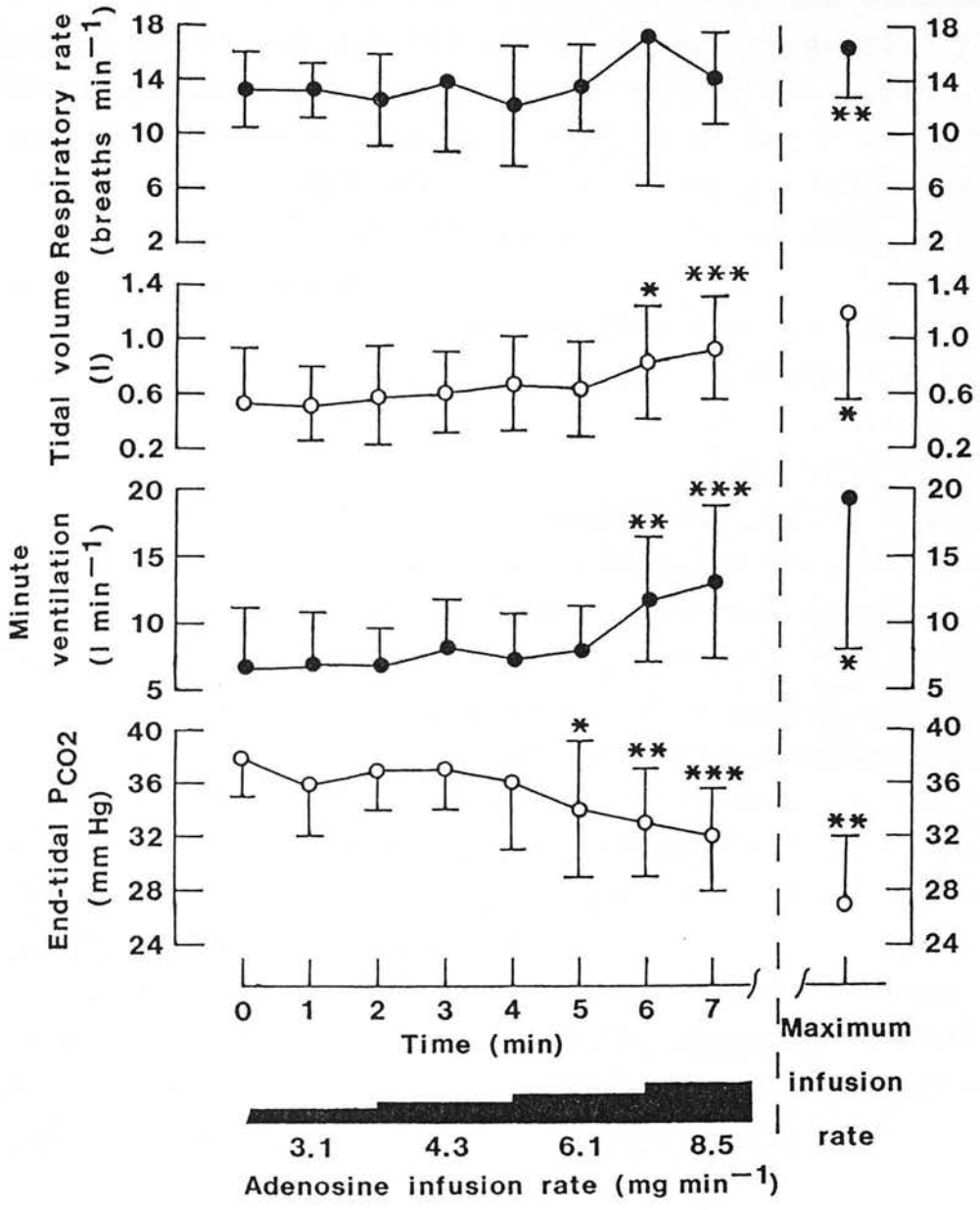

Fig. 3.1 - Changes in respiratory rate, tidal volume, minute ventilation and end-tidal PCO_2 during adenosine infusion. *P < 0.05; **P < 0.01; ***P < 0.001 for comparisons with baseline values. n = 6 except for minutes 1 and 2 where n = 5.

8.5 mg/min, and 19.2 (SD: 11.2) l/min at the maximum infusion rate ($P < 0.001$ and $P < 0.005$ respectively). These changes were predominantly due to an increase in tidal volume from 0.54 (SD: 0.39) l at baseline to 0.94 (SD: 0.36) l during adenosine infusion at 8.5 mg/min, and 1.20 (SD: 0.63) l at the maximum infusion rate ($P < 0.001$ and $P < 0.05$ respectively).

Respiratory rate at the maximum dose of adenosine, 16 (SD: 4) breaths/min, was significantly greater than the baseline value, 13 (SD: 3) breaths/min ($P < 0.01$). There were no other significant changes in respiratory rate.

The increase in minute ventilation during adenosine infusion was accompanied by an increase in V_T/T_I from 17 (SD: 10) l/min at baseline to 47 (SD: 21) l/min at the maximum infusion rate ($P < 0.05$). T_E fell from 3.1 (SD: 1.0) s at baseline to 2.4 (SD: 0.9) s at the maximum infusion rate ($P < 0.01$) but there was no significant change in T_I (mean: 1.7, SD: 0.5 s at baseline vs. mean: 1.5, SD: 0.5 s at the maximum infusion rate) or T_I/T_{TOT} (mean: 0.36, SD: 0.09 at baseline; mean: 0.39, SD: 0.07 at the maximum infusion rate).

$PETCO_2$ fell significantly from 38 (SD: 3) mmHg at baseline to 32 (SD: 4) mmHg during adenosine infusion at 8.5 mg/min, and 27 (SD: 5) mmHg at the maximum infusion rate ($P < 0.001$ and $P < 0.01$ respectively).

3.3.2 - CHANGES IN HEART RATE AND BLOOD PRESSURE DURING ADENOSINE INFUSION

Heart rate increased from 67 (SD: 7) beats/min at baseline to 86 (SD: 15) beats/min during adenosine infusion at 8.5 mg/min, and 105 (SD: 9) beats/min at the maximum infusion rate ($P < 0.01$ and $P < 0.001$ respectively) (Fig. 3.2). One subject developed a transient bradycardia of 30 beats/min with second degree heart block for a few beats during breath-holding immediately after discontinuing adenosine infusion (at 16.8 mg/min). His rhythm quickly reverted to sinus tachycardia. In no other subject was a bradycardia seen.

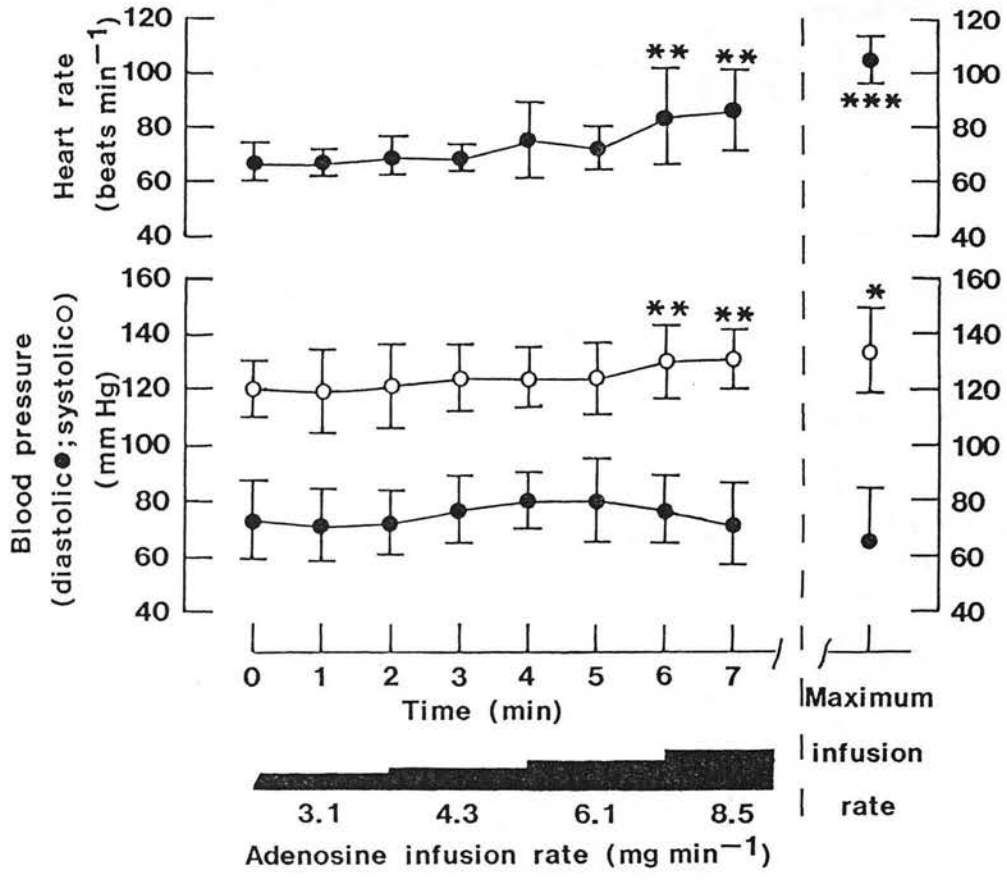

Fig. 3.2 - Changes in heart rate and blood pressure during adenosine infusion. Significance symbols as in Fig. 3.1. n = 6 except for minutes 1 and 2 where n = 5.

Systolic blood pressure increased significantly from 120 (SD: 10) mmHg at baseline to 131 (SD: 11) mmHg during adenosine infusion at 8.5 mg/min, and 134 (SD: 15) mmHg at the maximum infusion rate ($P < 0.01$ and $P < 0.05$ respectively). Diastolic blood pressure changed biphasically, increasing from 73 (SD: 14) mmHg at baseline to 80 (SD: 10) mmHg during adenosine infusion at 4.3 mg/min, with a subsequent fall to 66 (SD: 19) mmHg at the maximum infusion rate. These changes were, however, not statistically significant.

Significant hypotension did not occur during adenosine infusion at any dose used in this study.

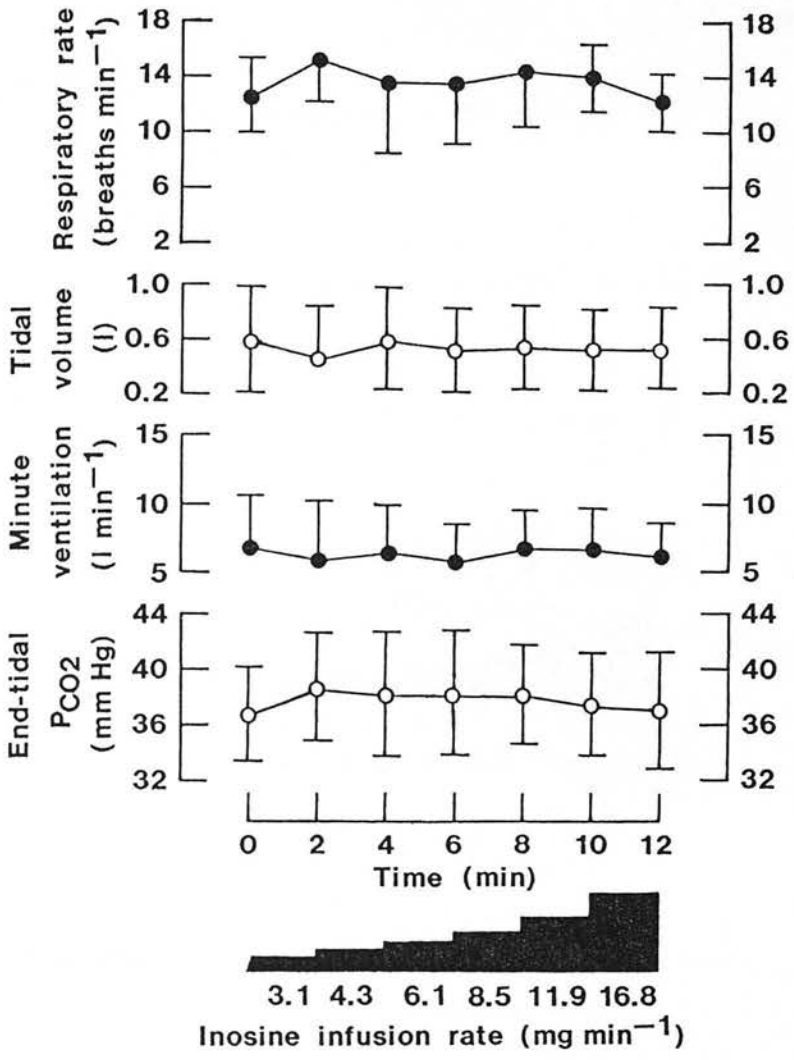

Fig. 3.3 - Respiratory rate, tidal volume, minute ventilation and end-tidal PCO_2 during inosine infusion. n = 6 except for minute 2 where n = 5.

3.3.3 - HAEMODYNAMIC AND RESPIRATORY VARIABLES DURING INOSINE INFUSION

In contrast to the changes observed during adenosine infusion, no significant changes in the above variables were observed during inosine infusion, despite the higher maximum infusion rate (16.8 mg/min) received by all subjects. Respiratory variables are shown in Fig. 3.3 and haemodynamic variables in Fig. 3.4.

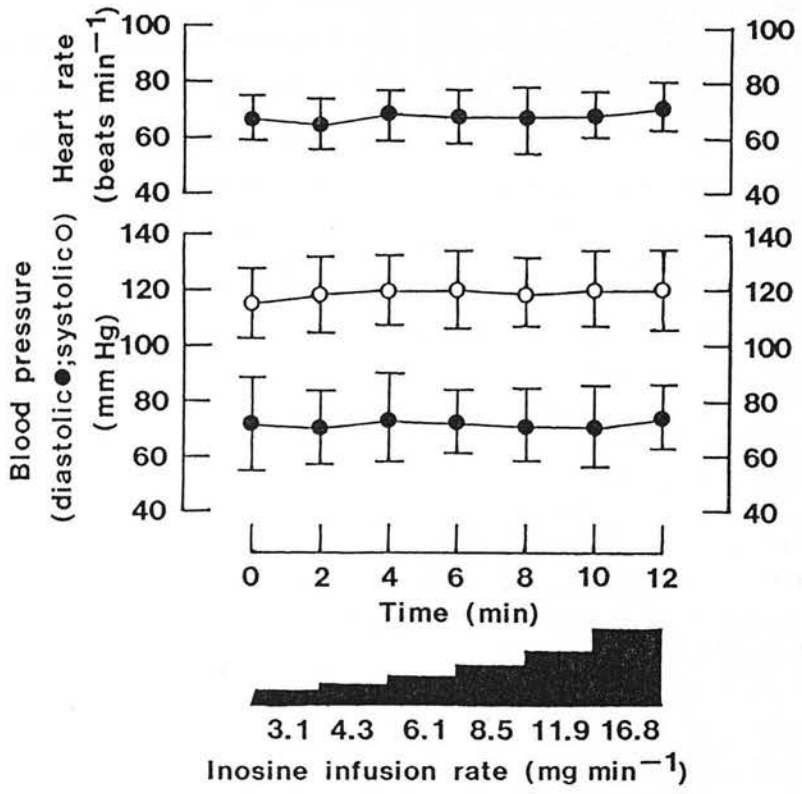

Fig. 3.4 - Heart rate and blood pressure during inosine infusion. n = 6 except for minute 2 where n = 5.

Results for V_T/T_I, T_I, T_E and T_I/T_{Tot} (baseline vs. infusion at 16.8 mg/min) were 21 (SD: 10) l/min vs. 19 (SD: 7) l/min, 1.5 (SD: 0.6) s vs. 1.7 (SD: 0.5) s, 3.4 (SD: 0.7) s vs. 3.3 (SD: 0.9) s, and 0.31 (SD: 0.08) vs. 0.34 (SD: 0.10) respectively (all changes not significant).

3.3.4 - SPIROMETRY

No subject reported wheeziness and spirometry showed no significant changes following either adenosine or inosine in the four subjects tested (Table 3.1).

Table 3.1 - Spirometry before and after adenosine and inosine infusion (best of 3 readings for each subject). Values are Mean (SD). n = 4

	Pre-Adenosine	Post-Adenosine	Pre-Inosine	Post-Inosine
PEFR (l/min)	635 (65)	594 (48)	572 (60)	588 (73)
FEV_1 (l)	4.25 (0.50)	4.10 (0.47)	4.12 (0.45)	4.19 (0.49)
FVC (l)	5.24 (0.63)	5.27 (0.56)	5.20 (0.72)	5.30 (0.63)

3.3.5 - SYMPTOMS

During adenosine infusion facial flushing was reported by all 8 subjects, dyspnoea by 7, throat discomfort by 5, epigastric discomfort by 4, headache by 4, paraesthesiae by 3 and retrosternal discomfort by 2. One subject was able to tolerate the maximum dose of adenosine infused (23.4 mg/min). In all other subjects the infusion was stopped at a lower dose rate because of the degree of dyspnoea and other sensations experienced. All sensations resolved within 1 to 2 min after stopping the infusion.

During inosine infusion one subject reported lightheadedness but all other subjects were asymptomatic.

3.4 - DISCUSSION

3.4.1 - CHANGES IN RESPIRATION DURING ADENOSINE AND INOSINE INFUSION

This study confirms the dose-dependent respiratory stimulant effect of adenosine in man, which was first

demonstrated as a transient phenomenon following intravenous boluses of adenosine (Watt & Routledge, 1985), and shows that ventilatory stimulation is sustained during infusion of the nucleoside. At the maximum infusion rate minute ventilation was trebled and mean $PETCO_2$ reduced by 11 mmHg. Several workers have reported similar observations (Maxwell et al., 1986; Fuller et al., 1987; Biaggioni et al., 1987).

As discussed in Chapter 1 adenosine is rapidly removed from the circulation principally by cellular uptake followed by metabolism. The present study has shown that intravenous inosine is without effect on respiration in the dose range studied. Therefore the respiratory stimulation produced by adenosine in this dose range does not depend on prior metabolism to inosine. These observations are supported by the findings of Norsted et al. (1987) that whereas intravenous and intracarotid injections of adenosine increased central inspiratory activity in anaesthetised cats inosine did not. Recently Lagerqvist et al. (1990) have also shown, using intravenous bolus injections, that inosine does not stimulate respiration nor cause chest discomfort such as can be caused by adenosine.

This study does not exclude the possibility that phosphorylation of adenosine is a prerequisite for its respiratory stimulant effect. However, as discussed in later chapters, there is considerable evidence that adenosine stimulates respiration at least in part by an action in the carotid bodies and it has been shown in the cat that a stable analogue of ATP does not stimulate the peripheral chemoreceptors (McQueen & Ribeiro, 1983). In addition studies in animals using long acting analogues of adenosine suggest that it acts via cell surface receptors of the A2 subtype in the carotid body (Monteiro & Ribeiro, 1987).

It has been suggested that useful information can be gained about the effects of ventilatory stimuli on the control of breathing by analysis of the component parts

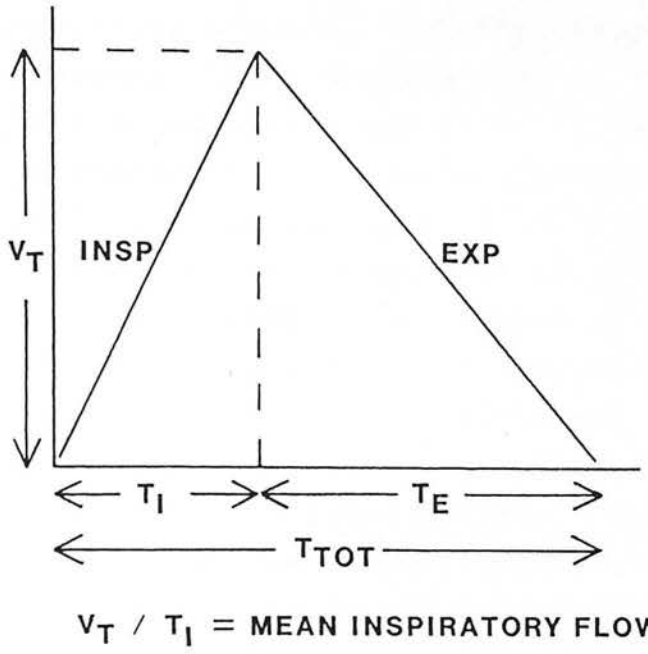

Fig. 3.5 - Diagram of a single respiratory cycle. Insp, inspiration; Exp, expiration. Other abbreviations as in text.

of ventilation.

Milic-Emili and Grunstein (1976) expressed minute ventilation in an equation of the form:

$$V = V_T \times f_R = 60 \times \frac{V_T}{T_I} \times \frac{T_I}{T_{TOT}} \qquad \text{(Eq. 3.1)}$$

In this analysis mean inspiratory flow, V_T/T_I is regarded as an index of "inspiratory drive" and T_I/T_{TOT} represents an index of "respiratory timing". V_T/T_I has been shown to be closely related to the level of chemical stimulation (Gardner, 1975; Newsom Davis & Stagg, 1975) and can provide a reasonable index of "inspiratory drive" provided inspiration starts at functional residual capacity and the mechanical properties of the respiratory system are fixed (Milic-Emili & Grunstein, 1976).

The changes observed in this study during adenosine infusion, namely an increase in V_T/T_I and a reduction in

T_E are qualitatively similar to those produced by a number of respiratory stimuli, including hypoxia and hypercapnia (Gardner, 1975; Remmers, 1976). We found no change in T_I during adenosine infusion. It has been suggested that whereas T_I may remain constant during hypercapnia until a threshold level of tidal volume is reached for termination of inspiration by Hering-Breuer reflexes (Clark & von Euler, 1972; Rebuck et al., 1976) there is a progressive shortening of inspiratory duration during progressive isocapnic hypoxia (Rebuck et al., 1976). Others, however, have found no consistent change in inspiratory duration during ventilatory stimulation by hypoxia, hypercapnia and exercise (Cunningham & Gardner, 1972; Jennett et al., 1974; Newsom Davis & Stagg, 1975). In any case a reduction in inspiratory duration when it occurs is considerably less than the reduction in expiratory duration. As shown by a later study (Chapter 5) functional residual capacity is probably increased by adenosine infusion, as it may be by hypoxia and hypercapnia, so mean inspiratory flow may not be a very reliable index of inspiratory drive in this situation.

3.4.2 - CHANGES IN HEART RATE AND BLOOD PRESSURE DURING ADENOSINE INFUSION

As discussed in Chapter 1 adenosine acts as a vasodilator in several vascular beds and both ATP, which is rapidly hydrolysed to adenosine in vivo, and adenosine have been used as hypotensive agents in man (Fukunaga et al., 1982b; Sollevi et al., 1984b). In the present study adenosine did not cause hypotension. On the contrary a slight, but statistically significant, increase in systolic blood pressure was observed. The observed respiratory stimulation is therefore not attributable to hypotension. A similar conclusion has been reached by others (Maxwell et al., 1986; Fuller et al., 1987; Biaggioni et al., 1987; Smits et al., 1987).

In healthy volunteers intravenous bolus injections of adenosine produce a biphasic heart rate response: an

initial transient bradycardia, probably due to a direct effect on the sinoatrial node as discussed in Chapter 1, followed by a more sustained tachycardia, probably reflex in origin (Watt & Routledge, 1986b). In the present study only an increase in heart rate was seen during adenosine infusion as has been reported by others (Biaggioni et al., 1986; Maxwell et al., 1986; Fuller et al., 1987). During sustained infusion lower concentrations of adenosine perfusing the sinus node and/or simultaneous activation of reflexes causing an increase in heart rate may explain the apparent absence of a negative chronotropic effect as seen after bolus injections. Possible mechanisms of the increase in heart rate will be discussed in Chapter 7.

3.4.3 - SPIROMETRY FOLLOWING ADENOSINE INFUSION

As discussed in Chapter 1 inhaled adenosine causes bronchoconstriction in asthmatic subjects, but not in normal subjects (Cushley et al., 1983). In the rat intravenous adenosine was shown to cause bronchoconstriction (Pauwels & Van Der Straeten, 1983). In the present study no subject reported a sensation of wheeze and in the four subjects in who spirometry was performed there were no significant changes. Biaggioni et al., (1986) similarly observed no change in spirometry in 8 subjects receiving adenosine infusion. Unlike the bronchoconstriction produced by inhaled adenosine in asthmatic subjects, which had not fully abated within 30 min (Cushley et al., 1983), the increased respiration produced by intravenous adenosine in the present study was observed to resolve within 1 min. These observations suggest that the respiratory stimulation is not secondary to airflow limitation. It is however possible that adenosine produces more subtle changes in airway calibre than could be detected by measurement of PEFR, FEV_1 and FVC and this question will be addressed further in later chapters.

3.4.4 - SYMPTOMS CAUSED BY ADENOSINE

In this study adenosine infusion was limited in all subjects except one by the development of various symptoms. Such effects may have contributed to the stimulation of ventilation. However Maxwell *et al*. (1986) observed stimulation of breathing at a dose of adenosine not associated with symptoms suggesting that they are not a necessary factor.

With regard to the question whether adenosine has a potential therapeutic rôle in the treatment of respiratory failure: the adverse subjective sensations noted in this study, in which I examined the dose-response relationship between ventilation and adenosine infusion rate up to the limit of each subject's tolerance, may not be relevant to longer term use of lower dose infusion of the nucleoside. If adenosine were to adversely affect airway calibre, this may be a limiting factor in patients with airways obstruction.

CHAPTER 4 - A COMPARISON OF THE EFFECTS OF INTRA-AORTIC INFUSION OF ADENOSINE PROXIMAL AND DISTAL TO THE CAROTID CIRCULATION.

4.1 - INTRODUCTION

Watt and Routledge (1985) suggested that adenosine might stimulate respiration by an action in the carotid bodies. The delay they observed between the injection of adenosine and the onset of respiratory stimulation was compatible with this suggestion. In support of this was the observation of McQueen and Ribeiro (1981) that adenosine increased afferent neural discharges from the carotid body of the cat. An action within the central nervous system was considered unlikely for the reasons discussed in Chapter 1. Involvement of a pulmonary reflex was thought to be unlikely because Gustafsson (1981) observed respiratory stimulation after injection of adenosine into the left ventricle of the rabbit. Nevertheless further work was clearly necessary to determine the site or sites of action of adenosine in man.

As discussed in Chapter 1 adenosine has a half-life in human blood measured in seconds. Therefore if adenosine-induced respiratory stimulation in man is mediated by the carotid bodies it would be expected that infusion of adenosine proximal to the carotid circulation would stimulate respiration whereas more distal administration would not. In this study the effects of such infusions were compared in patients with arterial catheters inserted for diagnostic purposes.

4.2 - METHODS

4.2.1 - SUBJECTS

The subjects were 12 patients (10 male) aged 46 to 67 (mean: 55) years and weighing 72 to 90 (mean: 74) kg (n = 10) scheduled to undergo cardiac catheterisation for investigation of chest pain (Table 4.1). A further

Table 4.1 - Patient characteristics.

Patient	Age (years)	Sex	Height (m)	Weight (kg)	Treatment	Diagnosis
1	60	M	-	-	B	No CAD
2	56	M	-	-	C N	1-VD
3	50	M	1.72	72	BC NA	1-VD
4	52	M	1.75	81	BC	1-VD
5	64	M	1.69	79	C	3-VD
6	60	M	-	77	BC N	3-VD
7	46	M	1.64	70	B N F	1-VD
8	46	M	1.82	90	N	2-VD
9	53	F	1.65	71	B D	1-VD
10	55	M	1.70	65	B	3-VD
11	67	F	-	57	B DN	1-VD
12	57	M	1.68	83	BC N	3-VD
Mean	55		1.71	74		
SD	7		0.06	10		

M, male; F, female; CAD, coronary artery disease; 1-VD, 1 vessel coronary artery disease; 2-VD, 2 vessel disease; 3-VD, 3 vessel disease; B, ß-adrenoceptor antagonist; C, calcium antagonist; D, diuretic; N, long-acting nitrate; A, aspirin; F, fenoprofen.

patient who agreed to participate was not included because a satisfactory position for the respiration transducer could not be found.

Eleven of the patients proved to have coronary disease. Careful consideration was given to the possibility of adverse cardiac events in such patients but it was considered that clinically significant adverse events were unlikely in view of the short half-life of adenosine.

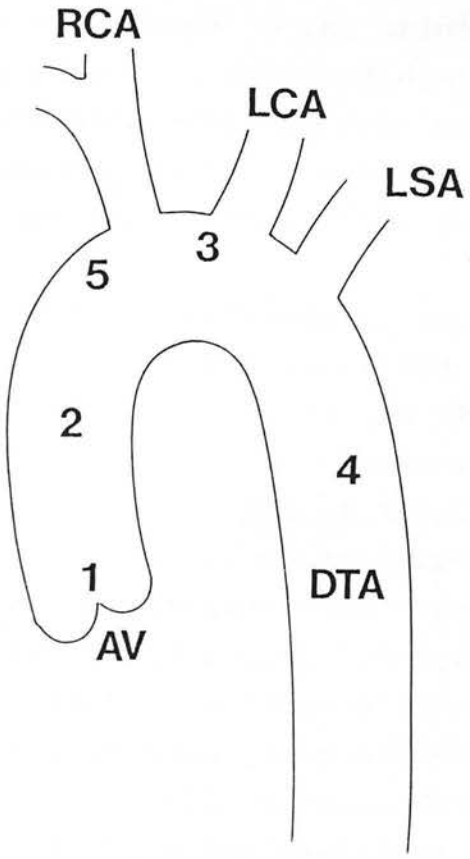

Fig. 4.1 - Sites of adenosine infusion. AV, aortic valve; RCA, right common carotid artery; LCA, left common carotid artery; LSA, left subclavian artery; DTA, descending thoracic aorta.

Prior to the study patients were receiving a variety of medications (Table 4.1). All therapy, other than sublingual nitrates, was stopped at least 12 h prior to the study but patients received premedication with diazepam 10 mg orally 1 hour prior to catheterisation.

4.2.2 - INFUSION PROTOCOL

Adenosine was administered by continuous intra-aortic infusion sequentially at five sites: (1) immediately above the aortic valve, (2) mid-ascending thoracic aorta, (3) top of the aortic arch, (4) mid-descending thoracic

aorta and (5) just proximal to the innominate artery (Fig. 4.1). Site 3 is close to the origin of the head and neck vessels and site 4 is situated distal to those vessels. Infusions were administered, prior to diagnostic angiography, through a pigtail catheter (8F) which had been inserted under local anaesthetic through the right femoral artery.

The initial rate of intra-aortic adenosine infusion was derived from the findings described in Chapter 1: an intravenous infusion rate of 6 mg/min of adenosine was sufficient to produce slight respiratory stimulation. A half-life of adenosine in blood of about 10 s (Klabunde, 1983) and a circulation time of 20 s from an antecubital vein to the aorta were assumed. The infusion rate at site 1 was therefore initially set at 1.6 mg/min, and was increased if necessary to produce a moderate increase in minute ventilation. In this group of patients the mean final dose was 2.9 (SD: 1.0) mg/min. Each infusion was thereafter continued at a constant rate while the cardiac catheter was moved at 1 min intervals from one site to the next. Patients were "blind" to the timing and direction of catheter movements.

4.2.3 - MEASUREMENTS

A single-lead electrocardiogram was recorded throughout the study. Respiration was recorded at 1 min intervals using a respiration transducer (Lectromed type 4320) and an Ormed MX216 recorder as described in Chapter 2. Intra-aortic pressure was measured in 8 subjects (nos. 3 to 11) via the cardiac catheter before and immediately after the adenosine infusion. In 4 patients (nos. 7, 8, 9 and 11) right femoral artery pressure was measured at 1 min intervals via the side-arm of an arterial sheath through which the cardiac catheter had been inserted. Patients were asked at 1 min intervals to report any symptoms.

4.2.4 - STATISTICAL ANALYSIS

Comparisons of minute ventilation, tidal volume, respiratory rate, heart rate (HR) and blood pressure (BP) at different sites were made using ANOVA and Student-Newman-Keuls test. Prior log transformation of minute ventilation and tidal volume data was performed. Blood pressure before and after the adenosine infusion was compared using Student's paired t-test. Changes in heart rate in patients receiving and in those not receiving ß-adrenoceptor antagonists were compared with the Mann-Whitney test. In one patient (no. 12) the study was stopped prematurely because of epigastric discomfort. Haemodynamic data were therefore analysed for the remaining 11 patients, but data on side-effects included all 12 patients. In one patient who completed the study (no. 8) the respiratory trace recorded at position 4 was of poor quality and could not be measured. Respiratory data are therefore presented for 10 patients.

4.3 - RESULTS

Respiratory, heart rate and blood pressure data are summarised in Table 4.2.

There was a significant difference in minute ventilation at different infusion sites ($P < 0.002$). Minute ventilation at baseline and site 4 did not differ from each other ($P > 0.5$) but were significantly less than minute ventilation at sites 1, 2 or 5 ($P < 0.025$ for each comparison). Minute ventilation at site 3 was intermediate and did not differ significantly from that at any other site. In 7 patients ventilation decreased as the catheter was moved from site 2 to site 3 and in 3 it increased, but in only one of these (no. 7) did the increase exceed 25%. There were no other significant inter-site differences in minute ventilation.

The difference in minute ventilation was attributable to inter-site differences in tidal volume ($P < 0.02$). Tidal volume during adenosine infusion at sites 1, 2 and 5 was higher than at baseline ($P < 0.05$ for each

Table 4.2 - Respiratory variables, heart rate and mean blood pressure during intra-aortic adenosine infusion at the sites described. Data are shown as mean (SD). RFA, right femoral artery. Other abbreviations as in text. The sites of infusion are in their anatomical order.

Variable	Baseline	Infusion Site				
		1	2	5	3	4
V (l/min) (n = 10)	5.3 (1.7)	8.8 (3.5)	10.1 (8.9)	9.7 (6.2)	6.8 (2.3)	5.1* (2.5)
f_R (breaths /min) (n = 10)	16 (4)	16 (4)	17 (5)	17 (3)	15 (2)	14* (3)
V_T (l) (n = 10)	0.35 (0.14)	0.57 (0.29)	0.60 (0.34)	0.62 (0.45)	0.46 (0.18)	0.38* (0.18)
HR (beats/min) (n = 11)	59 (9)	64 (13)	70 (15)	76 (17)	71 (14)	70 (14)
Mean RFA BP (mmHg) (n = 4)	92 (10)	94 (16)	99 (12)	102 (13)	94 (14)	92 (12)

*n = 9

comparison). Tidal volume at site 4 did not differ from baseline ($P > 0.5$), but approached a significant difference from sites 1, 2 and 5 ($0.05 < P < 0.1$ for each comparison). There was no significant difference in respiratory rate at different infusion sites.

There was also a significant difference in heart rate at different infusion sites ($P < 0.001$). Heart rate at baseline and at site 1 was significantly less ($P < 0.01$ for each comparison) than heart rate at sites 2, 3,

Table 4.3 - Symptoms reported during intra-aortic adenosine infusion at the sites described. The sites of infusion are in their anatomical order. n = 12.

Symptom	Site					Total no. Patients
	1	2	5	3	4	
Dyspnoea	5	6	1	5		8
Chest discomfort	1			4	6	8
Epigastric discomfort	1	1		2	7	8
Flushing	1	5	3	1		6
Dizziness			2	3		5
Headache	1	3	2			5
Throat, neck & face discomfort	1	1	3	2		5

4 or 5 which were not significantly different from one another. In the 3 patients who were not receiving ß-adrenoceptor antagonists the increase in heart rate (mean: 30, range: 19 to 38 beats/min) was significantly greater than the increase (mean: 12, range: 8 to 16) in those patients who were receiving such drugs ($P = 0.02$).

In the eight patients in whom mean intra-aortic pressure was measured before and immediately after adenosine infusion, there was no significant change (mean: 89, SD: 12 mmHg before vs. mean: 84, SD: 12 mmHg following adenosine). In the four patients in whom right femoral artery pressure was measured at each infusion site no significant changes were demonstrable but the

small sample size may have allowed a Type II statistical error.

Adverse effects were reported by a number of patients during adenosine infusion but these were mild with one exception. That patient (number 4), who had a symptomatic hiatus hernia, experienced epigastric pain during adenosine infusion at site 3 and the study was promptly terminated at the patient's request. No other subject requested that the study be terminated (although all were aware that they might do so). Other symptoms and their relationship to the site of infusion are shown in Table 4.3. All symptoms resolved within 60 s of stopping the adenosine infusion.

4.4 - DISCUSSION

This study confirmed with an intra-aortic infusion the respiratory-stimulant effect of adenosine in man previously observed with intravenous administration (Watt & Routledge, 1985; Maxwell *et al.*, 1986; this thesis Chapter 3). Further the results demonstrate that the respiratory-stimulant effect of intra-aortic adenosine depends on the site of administration. Adenosine infused distal to the carotid circulation (site 4) caused no respiratory stimulation, but infusion of adenosine proximal to the carotid circulation caused respiratory stimulation both before (sites 1 and 2) and after (site 5) more distal adenosine infusion.

4.4.1 - SITE OF ACTION OF ADENOSINE

These findings raise the question of why adenosine-induced respiratory stimulation should depend on perfusion of the carotid circulation. The effect could be mediated by the carotid bodies or brain chemoreceptors. The latter alternative appears unlikely because, as already discussed (Chapter 1), adenosine and its long-acting analogues depress respiration when applied locally to the brain. Buss *et al.* (1986) found that adenosine caused respiratory stimulation in the rabbit

and that the effect was abolished by bilateral division of the afferent nerve supply of the carotid bodies. Monteiro & Ribeiro (1987) demonstrated the same phenomenon in the rat. These observations suggest that adenosine stimulates respiration by an action on the carotid body in those species.

The present data support but do not prove the hypothesis that adenosine-induced respiratory stimulation in man is also carotid body mediated. Further support for this hypothesis and also for the suggestion (Watt & Routledge, 1985) that adenosine may be a physiological mediator or modulator of the ventilatory response to hypoxia was provided by Maxwell et al. (1986). These authors reported that adenosine infusion in normal subjects potentiated the ventilatory response to hypoxia, a response mediated in man by the carotid bodies (Lugliani et al., 1971). Furthermore Griffiths et al. (1990) recently showed that adenosine did not stimulate respiration in 2 patients whose ventilatory response to hypoxia was depressed following carotid endarterectomy. In view of the evidence that separate mechanisms subserve the responses to hypoxia and hypercapnia in the carotid body (Eyzaguirre, 1984) it is of interest that Maxwell et al. found that adenosine had no effect on the ventilatory response to hypercapnia.

The mechanisms by which changes in blood gas tensions and pH alter neural discharges from the carotid bodies (chemotransduction) remain to be elucidated (Eyzaguirre, 1984). Although expressed in various forms, the "metabolic hypothesis" proposes that a decrease in intracellular ATP in the carotid bodies during hypoxia results in the release from Type 1 (glomus) cells of a transmitter which excites synaptically connected fibres of the carotid sinus nerve (Eyzaguirre, 1984). Obeso et al. (1985) showed that the ATP content in the cat carotid body falls during hypoxia, although Acker et al. (1984) had previously observed no change. To my knowledge the concentration of adenosine in the carotid body has not

been measured in any species. However in view of the weight of evidence from other tissues studied (see Chapter 6) it seems very likely that the interstitial adenosine concentration in the carotid body should increase when there is increased breakdown of ATP, e.g. during hypoxia. It remains to be shown whether such an increase in adenosine concentration would participate in the mechanism(s) of chemotransduction, but given the abundant evidence of neuromodulation by adenosine in other tissues (see Chapter 1) this is an attractive hypothesis. Since adenosine generally inhibits neurotransmission, any participation of the nucleoside in chemotransduction is likely to involve a complex interaction with other neurally active substances.

The bronchial arteries arise most commonly from the antero-lateral aspect of the descending thoracic aorta ½ to 1 inch distal to the left subclavian artery (Marchand et al., 1950), although they may arise from: lower in the thoracic aorta, the inferior aspect of the aortic arch, the first right aortic intercostal artery and occasionally the right internal mammary artery (Romanes, 1968; Harris & Heath, 1986). Therefore the possibility that site 5 was distal to the origin of the bronchial arteries in some patients cannot be excluded. For this reason it is possible that an increased adenosine concentration perfusing the lungs via the bronchial arteries during adenosine infusion into the aorta at sites 1,2 and 5 caused the observed changes in respiration by an intra-pulmonary effect. However if that were the case an increase in ventilation might have been expected in the majority of patients as the catheter tip was moved from site 2 to site 3 which is nearer to the most likely origin of the bronchial arteries.

Whether adenosine might have stimulated respiration via an effect on the baroreceptors is uncertain. Hypotension can stimulate respiration via a baroreflex effect (Heistad et al., 1975). However no fall in blood pressure was observed in this study. Since adenosine

usually relaxes vascular smooth muscle any local effect might be expected to cause changes in wall stretch in the carotid sinus opposite to those caused by hypotension and therefore unlikely to stimulate ventilation. However a direct depressant effect of adenosine on neural discharges from the carotid sinus, mimicking the effect of hypotension, cannot be excluded.

4.4.2 - EFFECTS OF ADENOSINE ON HEART RATE AND BLOOD PRESSURE

The pattern of site-dependent changes in heart rate differed from that of minute ventilation. Heart rate increased after adenosine infusion was started; the increase over baseline values being apparent during infusion at sites 2, 3, 4 and 5. There was no fall in blood pressure to suggest that a baroreceptor reflex mediated the changes in heart rate. It is not clear why heart rate did not increase at site 1. It is possible that a sufficiently high concentration of adenosine was perfusing the coronary circulation, to exert a negative chronotropic effect on the sinoatrial node (see Chapter 1) thereby opposing any increase in heart rate produced by other mechanisms.

The increase in heart rate seen at other sites may have been secondary to the increase in ventilation similar to the response elicited by hypoxic stimulation of the carotid body observed in the dog (Daly & Scott, 1958). If this is the case it is unclear why the changes in heart rate show a pattern different from the respiratory changes. Heart rate does not return rapidly to baseline following boluses of adenosine (Watt & Routledge, 1986b) so it is possible that in the present study there was insufficient time during the period of infusion at site 4 for a return to baseline heart rate to occur. Alternatively a reflex secondary to the adverse effects or stimulation of other peripheral receptors may have contributed to the increased heart rate and hence its persistence during infusion at site 4 where some

adverse effects were more common.

Eight of the 11 patients who completed the study were receiving treatment with ß-adrenoceptor blockers and this seems to have reduced the magnitude of the heart rate changes in those patients. Effects of adenosine on heart rate will be discussed further in Chapters 7 and 8.

Measurement of intra-aortic blood pressure before and immediately after the adenosine infusions revealed no significant change. However it is possible that because of the short half-life of adenosine the post-infusion measurement was too late to detect a change. Nevertheless no change was seen in the 4 patients in whom blood pressure was monitored throughout, confirming the finding in the previous study that adenosine-induced respiratory stimulation is not due to hypotension. There was a trend in these 4 patients for blood pressure to be higher when adenosine was infused proximal to the carotid circulation. This was not significant but numbers were small and a Type II statistical error is therefore possible.

4.4.3 - SYMPTOMS CAUSED BY ADENOSINE

It has been proposed that exogenous adenosine produces transient chest discomfort by directly stimulating afferent cardiac nerves, and that adenosine released spontaneously during cardiac ischaemia might contribute to the symptom of angina pectoris (Sylvén et al., 1986). The present observations provide information on the site of origin of adenosine-induced chest discomfort. In seven of the eight patients reporting such discomfort, it occurred during adenosine infusion at sites 3 or 4. In view of the short half-life of adenosine in human blood it is unlikely that such chest sensations in those patients are cardiac in origin. In the single patient who reported "chest tightness" during adenosine infusion at site 1 but at no other site, the heart might have been the source of adenosine-induced discomfort.

Adenosine reproduces the epigastric pain of duodenal

ulceration (Watt et al., 1987b). While epigastric discomfort of some degree occurred in 8 patients in this study, this was only marked in the patient with a hiatus hernia. It may be, therefore, that not only duodenal ulceration but also other inflammatory lesions of the upper gastrointestinal tract may predispose to adenosine-induced epigastric discomfort by mechanisms as yet undefined. Adenosine-induced pain will be discussed further in Chapter 7.

The finding in this study that some symptoms were more common during adenosine infusion at site 5 (Table 4.3) and that minute ventilation was no different from the baseline value at that time suggests that symptoms do not play a large rôle in causing the ventilatory stimulation produced by adenosine.

CHAPTER 5 - ANTAGONISM OF THE EFFECTS OF ADENOSINE INFUSION BY AMINOPHYLLINE

5.1 - INTRODUCTION

As discussed in Chapter 1 cell-surface adenosine receptors of at least 2 classes have been described. Methylxanthines such as theophylline have been shown to act as competitive antagonists at these receptors and therefore are useful tools for investigating the possible sites of action of adenosine in mediating its biological effects. Antagonism by such a methylxanthine suggests that an effect of adenosine is probably mediated by cell-surface receptors. The purpose of the study described in this chapter was to examine the effect of aminophylline (theophylline ethylenediamine) on the responses to adenosine infusion, to test the hypothesis that the direct effects of adenosine are mediated by stimulation of cell-surface receptors.

Since adenosine-induced respiratory stimulation causes reduced end-tidal and therefore arterial PCO_2, the observed effects of adenosine on minute ventilation during sustained infusion will not fully reflect the magnitude of the ventilatory stimulus because of antagonistic effects of reduced $PaCO_2$ and increased pH on the peripheral and central chemoreceptors. This problem could be obviated by increasing the CO_2 content of the inspired gas as necessary to maintain end-tidal PCO_2 constant. However this would entail the use of a mouthpiece or facemask with the attendant disadvantages discussed in Chapter 2. In this study an intermittent adenosine infusion protocol was used, with recovery periods between infusions at each dose, to minimise the effects of alterations in blood gas tensions and pH. It was hoped that the peak minute ventilation during each infusion would reflect the ventilatory stimulus by adenosine better than data obtained with a continuous step-wise infusion protocol. However it was recognised that increased ventilation can alter blood gas tensions

rapidly so the effects of such alterations could not be completely prevented with this approach. Use of an intermittent infusion protocol would also facilitate substitution of adenosine by placebo at any point in the protocol.

A full understanding of the mechanisms of the observed cardiovascular and respiratory effects of exogenous adenosine and the possible relevance of these effects could only come with an understanding of the concentrations of adenosine producing these effects at its sites of action. Measurement of plasma adenosine concentrations was considered the first step in providing that understanding. In this study changes in the peripheral venous plasma concentration of adenosine during exogenous infusion were measured. However it was recognised that, because of the short half-life of adenosine in blood, plasma and interstitial concentrations of adenosine were unlikely to be identical and also that plasma concentrations at steady state might differ at different sites in the circulation. Nevertheless it was considered that changes in venous plasma adenosine concentrations might provide a useful index and lower limit of changes elsewhere.

5.2 - METHODS

5.2.1 - SUBJECTS AND INFUSIONS

The subjects were 10 normal volunteers (9 male), aged 23 to 55 years (mean: 33) and weighing 55 to 89 kg (mean: 71, SD: 12) (Appendix 3). A single patient known to suffer from asthma also completed the same protocol, as a pilot study of the effects of adenosine infusion in such patients.

Each subject was studied on two occasions which are referred to subsequently in this chapter as the "placebo leg" and the "aminophylline leg". Adenosine was administered on each occasion after prior infusion over 10 min, in randomised single-blind manner, of either

aminophylline (6 mg/kg in total volume of 30 ml sterile water) or placebo (30 ml of 0.9% sodium chloride). These initial infusions are referred to in this discussion as "pretreatment". Aminophylline was preferred to theophylline alone since it is more soluble and is the preparation of theophylline for injection normally used in clinical practice. The dose of aminophylline was chosen on the basis of previous work suggesting that a loading dose of 6mg/kg is likely to cause peak serum concentrations of 10 μg/ml, i.e. in the "therapeutic range", with a low likelihood of causing serious toxicity (Hendeles et al., 1978). In view of the expected duration of each study (no more than 2½ hours for the adenosine infusions) and the reported half-life of theophylline of approximately 4 to 9 h (Hendeles et al., 1978) a continuous maintenance infusion of aminophylline was not considered necessary.

Adenosine was infused in 5 min stages at each dose with recovery periods of at least 15 min between stages. Adenosine was given at an initial rate of 2.3 mg/min with increments, until limited by symptoms, to 4.3, 8.5, 11.9, 16.8 and 23.4 mg/min. A control infusion (0.9% sodium chloride) was also given, single blind, instead of adenosine usually after at least one adenosine infusion stage causing definite subjective and objective changes. The protocol for the infusions and the measurements made is illustrated in Fig. 5.1.

5.2.2 - MEASUREMENTS AND SAMPLES

A single-lead electrocardiogram (ECG) was recorded throughout and used to determine heart rate. Arterial blood pressure was recorded using an electrical sphygmomanometer (Programmed Electrosphygmomanometer PE-300, Narco Biosystems Inc., Houston, Texas). This had an internal calibration of 100 mmHg which was found to be accurate to within ± 10% by comparison with a mercury manometer. Respiration was recorded using a respiratory inductance plethysmograph as described in Chapter 2.

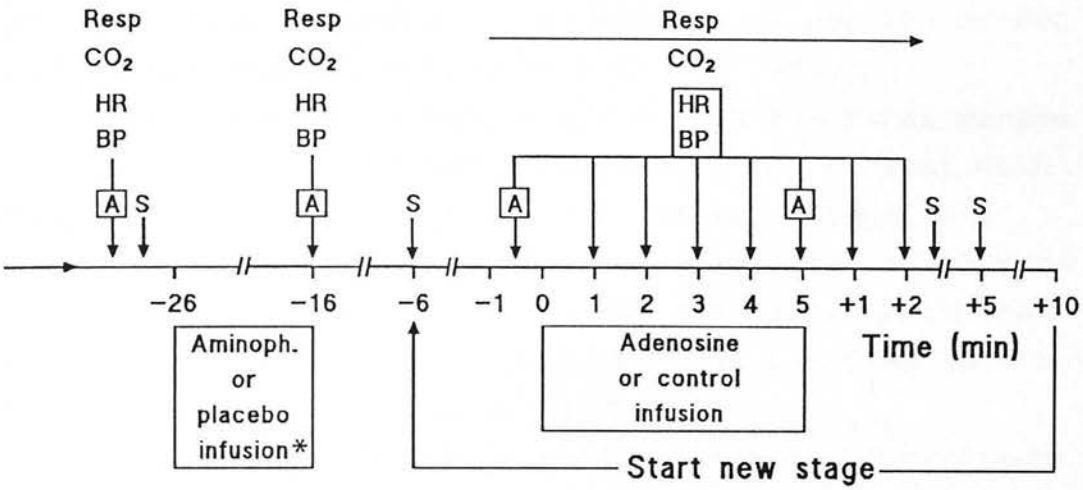

Fig. 5.1 - Protocol for the infusions and measurements made. Resp, respiration; CO_2, end-tidal PCO_2; HR, heart rate; BP, blood pressure; A, plasma adenosine concentration; S, spirometry; Aminoph., aminophylline. * indicates the "pretreatment".

During the study exhaled gas was sampled continuously by a fine bore catheter taped to each subject's cheek with its tip just inside the left nostril and PCO_2 was measured using a high speed response CO_2 analyser (Type 901 MK 2. P.K. Morgan Ltd.). The respiratory and PCO_2 traces were recorded on a chart recorder (Rikadenki). Respiratory variables and $PETCO_2$ were subsequently derived from the traces obtained. Mean values for 40 s periods at each time point were used for analysis.

Spirometry was performed using a rolling seal spirometer (Vitalograph Ltd) connected to an Apple IIc microcomputer which utilised a programme (Spirotrac II) to process the signal and derive various indices of the expiratory flow volume curve which were recorded by a

printer. The mean differences between values obtained from this programme and values obtained manually from the spirometer tracings were found to be -0.02 (SD:0.05) l, -0.003 (SD: 0.04) l and -0.10 (SD: 0.19) l/s for FEV_1, FVC and forced expiratory flow between 25 and 75% of FVC ($FEF_{25-75\%}$) respectively (n = 9 in each case).

Blood was sampled before and during the final minute of each stage in 6 subjects (including the patient with asthma) for measurement of venous plasma adenosine concentrations. These samples were drawn from a separate intravenous plastic cannula in the arm opposite to that used for infusions. The method of blood sampling and the assay used are described in Chapter 2.

Blood samples (5 ml) were also taken for measurement of serum theophylline concentrations just before the first adenosine/control infusion stage (15 min following the aminophylline or placebo pretreatment) and following the final stage. Assays for theophylline were kindly performed by Mr. D. C. Buss using a reversed phase HPLC method which has previously been described (Buss, 1990).

Subjects were asked to report any symptoms every minute during and for 2 min after each stage.

5.2.3 - STATISTICAL ANALYSIS

For baseline values comparisons were made of values for different infusion stages, rather than infusion rates, to facilitate detection of any trends during the study, e.g. due to aminophylline. All subjects received at least five infusion stages (including the control infusion) during each leg. Therefore baseline values for the first 5 infusion stages were compared with values before and after the pretreatment by ANOVA. Values before the pretreatment were also compared with values before the final infusion stage for each subject by Student's paired t test.

All subjects received adenosine at infusion rates up to at least 8.5 mg/min. Therefore for changes during infusions data for the control infusion and adenosine

infusions at 2.3, 4.3, 6.1 and 8.5 mg/min were also compared by ANOVA. Data for the control infusion and adenosine infusion at the maximum dose for each subject were also compared using the paired t test.

To simplify the analysis the peak minute ventilation and the trough $PETCO_2$ for each subject during each infusion were used for these comparisons. For V_T and f_R the values at the time of peak minute ventilation were used. Since previous work had shown an increase in heart rate and systolic blood pressure and a decrease in diastolic blood pressure during adenosine infusion the peak heart rate and systolic blood pressure and trough diastolic blood pressure were used. Data for minute ventilation were log-transformed prior to analysis.

The slopes of dose-response curves obtained after placebo and aminophylline pretreatment were compared using the following method. For every subject for any given variable the differences between the values obtained following aminophylline and placebo pretreatment at each dose of adenosine up to and including 8.5 mg/min were calculated. ANOVA was then performed. The sum of squares for doses (S_D) can be regarded as consisting of two components, S_{DL} and S_{DR}, where S_{DL} relates to the difference due to a linear increase (or decrease) with dose and S_{DR} to the difference not accounted for by the linear change. Since:

$$S_D = S_{DL} + S_{DR} \qquad \text{(Eq. 5.1)}$$

it follows that:

$$S_{DR} = S_D - S_{DL} \qquad \text{(Eq. 5.2)}$$

S_{DL} can then be calculated from:

$$S_{DL} = \frac{L^2}{A} \qquad \text{(Eq. 5.3)}$$

(Armitage & Berry, 1987) where:

$$(1)\ L = \sum_{i=1}^{5} \bar{y}_i (d_i - \bar{d}) \qquad \text{(Eq. 5.4)}$$

where 5 is the number of doses, y_i are the differences between responses during the placebo and aminophylline leg for each subject at dose level i, $\bar{y}_i$ is the mean value for all the subjects at dose level i and $\bar{d}$ is the mean dose given.

$$(2)\quad A = \frac{1}{n} \times \sum_{i=1}^{5} (d_i - \bar{d})^2 \qquad \text{(Eq. 5.5)}$$

where n is the number of subjects (in this case 10).
A test for the linear dose-response comes from comparing S_{DL}/Residual Mean Square with an F-distribution on 1 and (residual df) degrees of freedom.

To simplify the analysis of changes in spirometric variables so that any changes occurring over the course of each study or acutely following adenosine infusion would be detected data for the following times were compared by analysis of variance: before and following the pretreatment, following adenosine infusion at 2.3 and 4.3 mg/min (since all subjects received at least these two doses before the control infusion), following the control infusion and following adenosine infusion at the maximum dose (which in all subjects was after the control infusion).

For analysis of changes in FRC (see below) the means of the values during adenosine infusion, i.e. minutes 1 to 5, were calculated for each subject. The resultant values for the control infusion and adenosine infusion at the maximum dose were compared using the paired t test.

5.3 - RESULTS

Full results are presented for the 10 normal subjects. The analysis of changes in venous plasma adenosine concentrations includes values from the single subject with asthma. Since this subject developed bronchoconstriction, which is important, during adenosine infusion results of his spirometric tests are also shown.

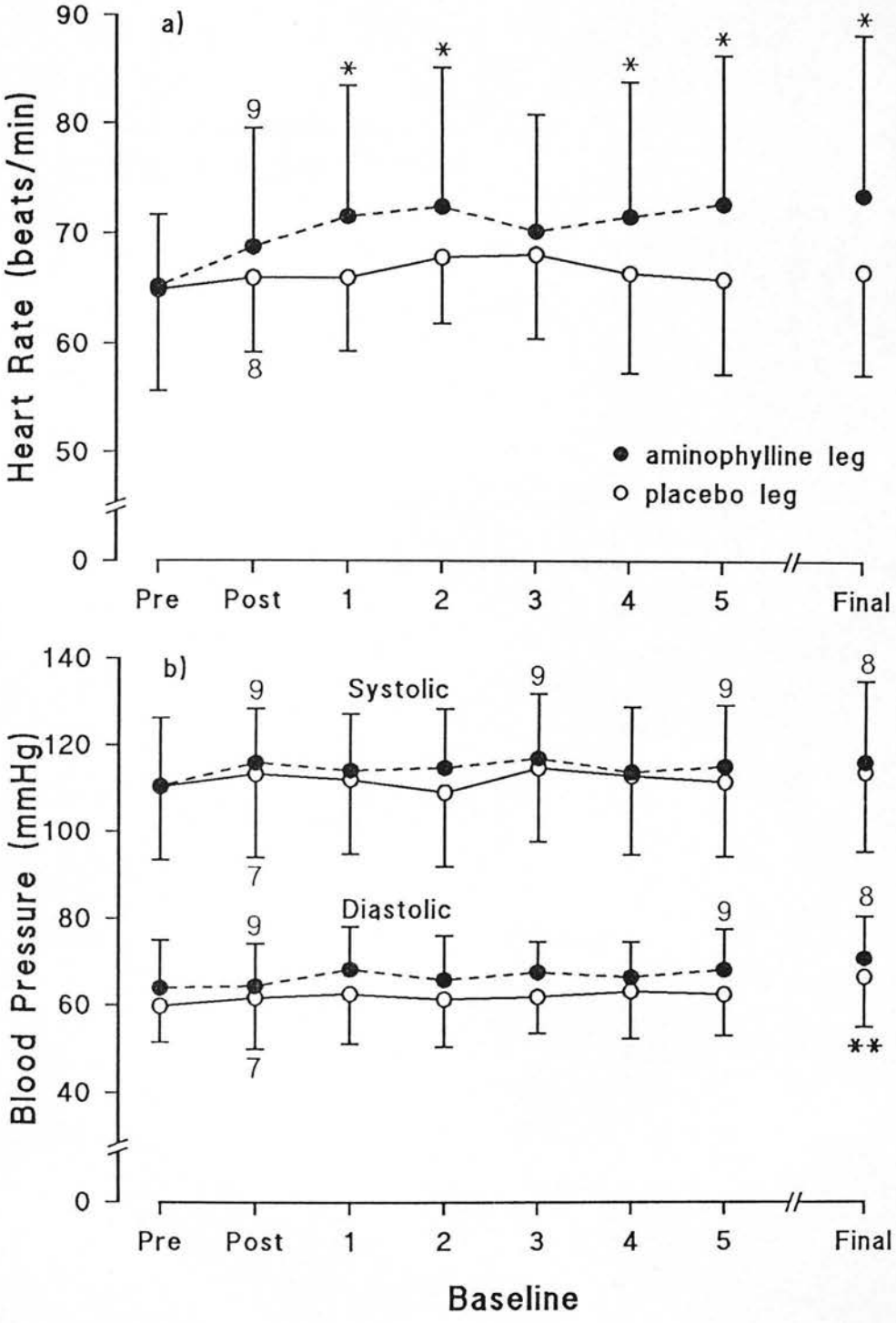

Fig. 5.2 - Baseline heart rate and blood pressure at the time points and for the infusion stages shown.

Pre, prior to the pretreatment; Post, immediately following the pretreatment; Final, the final infusion stage for each subject on the respective leg. Baselines 1 to 5 represent the first five infusion stages (including control in most cases). $^{*}P < 0.05$; $^{**}P < 0.01$ for comparison with the "Pre" values. n = 10 except where shown.

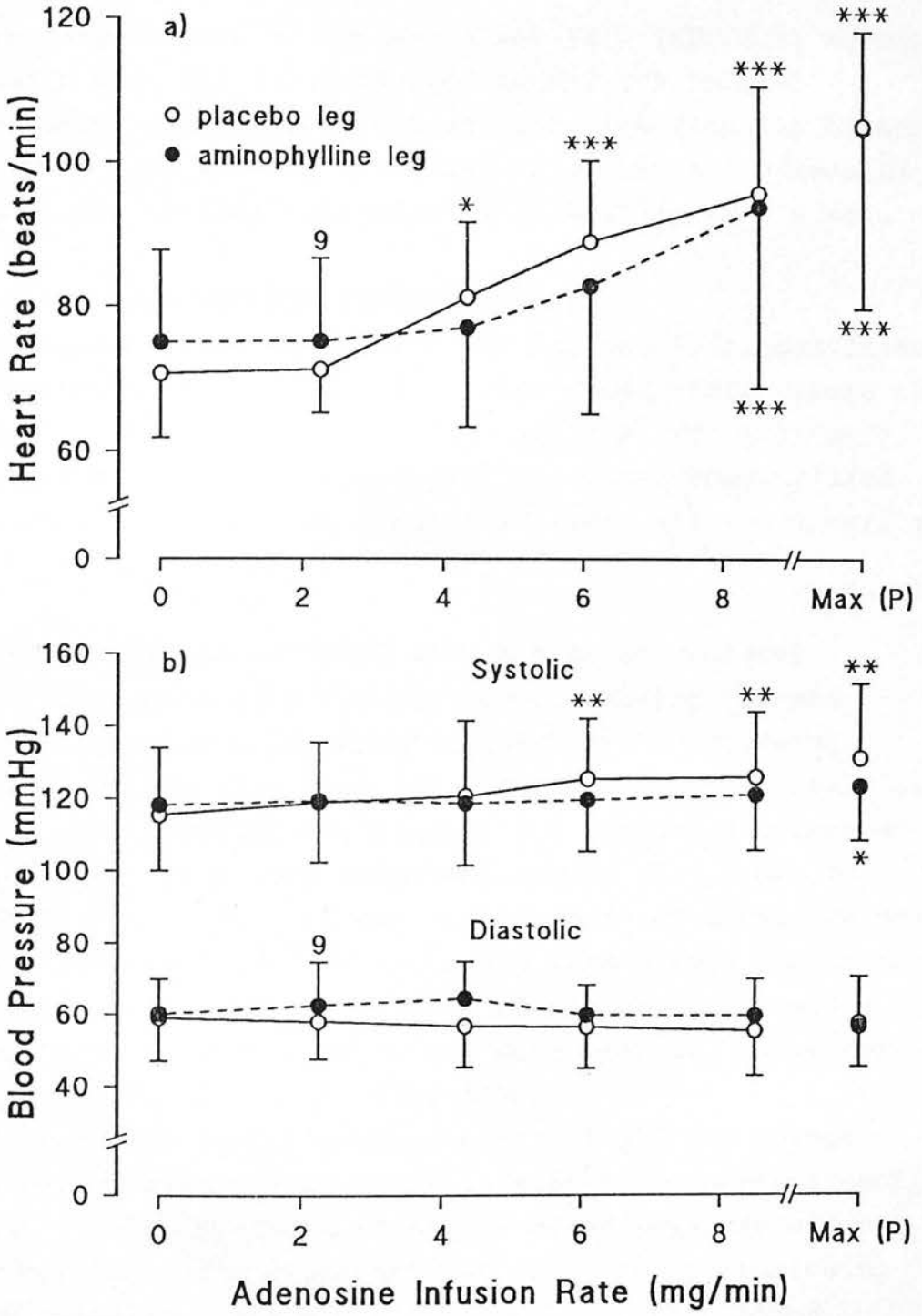

Fig. 5.3 - Effect of aminophylline on changes in heart rate and blood pressure during adenosine infusion. Max (P), the maximum dose received by each subject following placebo pretreatment. 0 mg/min represents the control infusion. *P < 0.05; **P < 0.01; ***P < 0.001 for comparisons with control. n = 10 except where shown.

5.3.1 - DOSE OF ADENOSINE

The maximum dose of adenosine was 12.3 (SD: 3.4) mg/min (mean: 175, SD: 41 μg/kg/min; range: 109 to 236) following placebo pretreatment and significantly higher at 15.6 (SD: 4.0) mg/min (mean: 224, SD: 61 μg/kg/min; range 109 to 330) following aminophylline ($P < 0.01$).

5.3.2 - THEOPHYLLINE CONCENTRATIONS

The theophylline concentrations following aminophylline infusion were 10.4 (SD: 2.1) mg/l immediately before the first adenosine infusion stage and 7.8 (SD: 1.3) mg/l following the final stage ($n = 9$). Basal theophylline concentrations on the placebo leg were all < 0.6 mg/l ($n = 9$).

5.3.3 - CHANGES IN HEART RATE AND BLOOD PRESSURE

Baseline heart rate did not vary following placebo pretreatment but increased significantly following aminophylline ($P < 0.05$ for ANOVA and paired t analyses; Fig. 5.2a). During the placebo leg adenosine increased heart rate in a dose-dependent manner ($P < 0.001$ for ANOVA; Fig. 5.3a). At the maximum dose of adenosine mean peak heart rate was 35 beats/min higher than during the control infusion ($P < 0.001$). Aminophylline caused a significant flattening of the dose-response curve for this effect ($P < 0.05$; Fig. 5.3a).

Baseline systolic blood pressure did not change following either placebo or aminophylline pretreatment (Fig. 5.2b). Baseline diastolic blood pressure did not change following aminophylline (Fig. 5.2b). Following placebo pretreatment the baseline diastolic blood pressure before infusions 1 to 5 did not change significantly but before the final infusion stage the mean diastolic blood pressure, 67 mmHg, was significantly higher than the value, 60 mmHg, before the placebo infusion ($P < 0.002$; Fig. 5.2b).

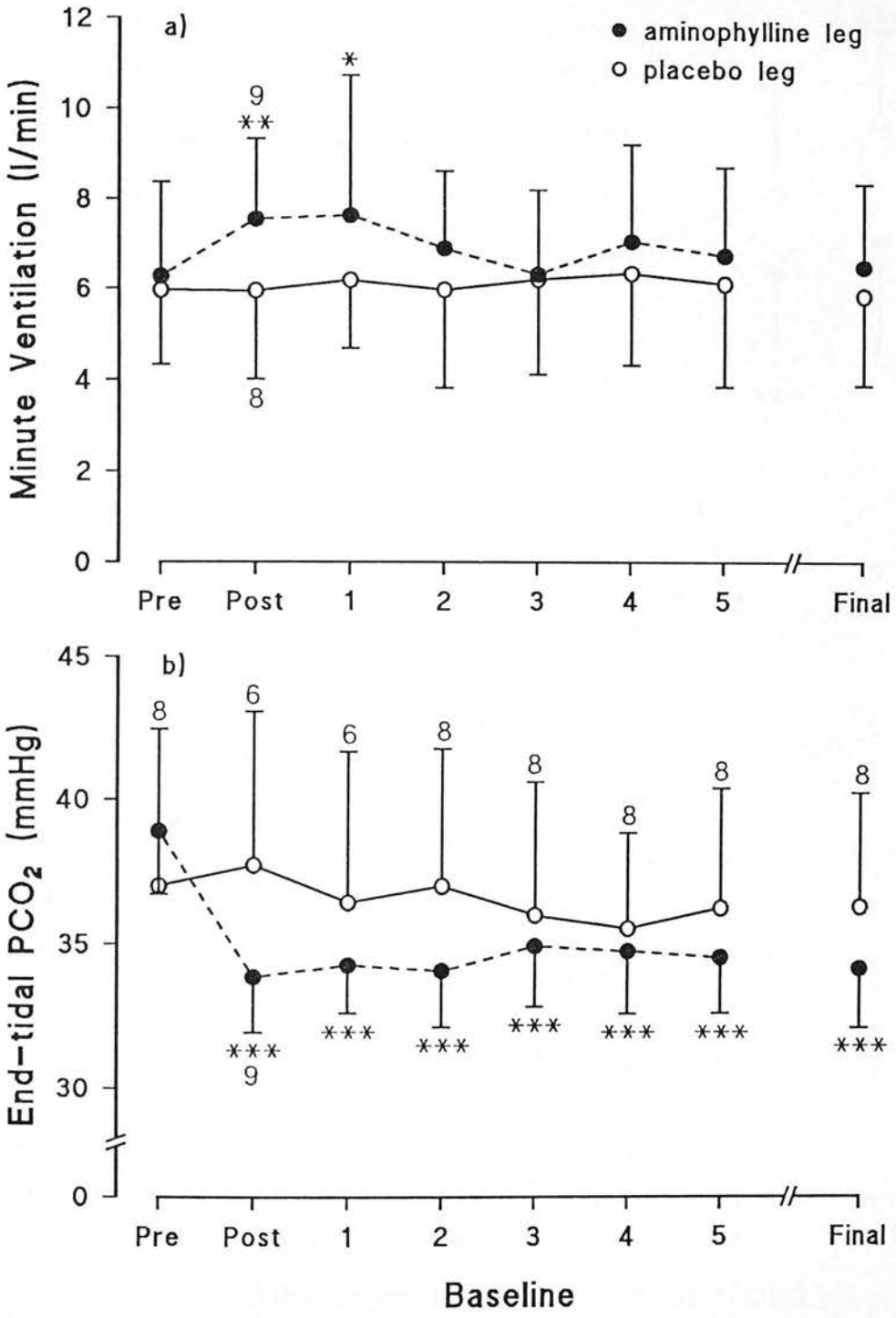

Fig. 5.4 - Baseline minute ventilation and end-tidal PCO_2 at the time points and for the infusion stages shown. Abbreviations as in Figs. 5.2 and 5.3. *P < 0.05; **P < 0.01; ***P < 0.001 for comparisons with values before the pretreatment ("Pre"). n = 10 except where shown.

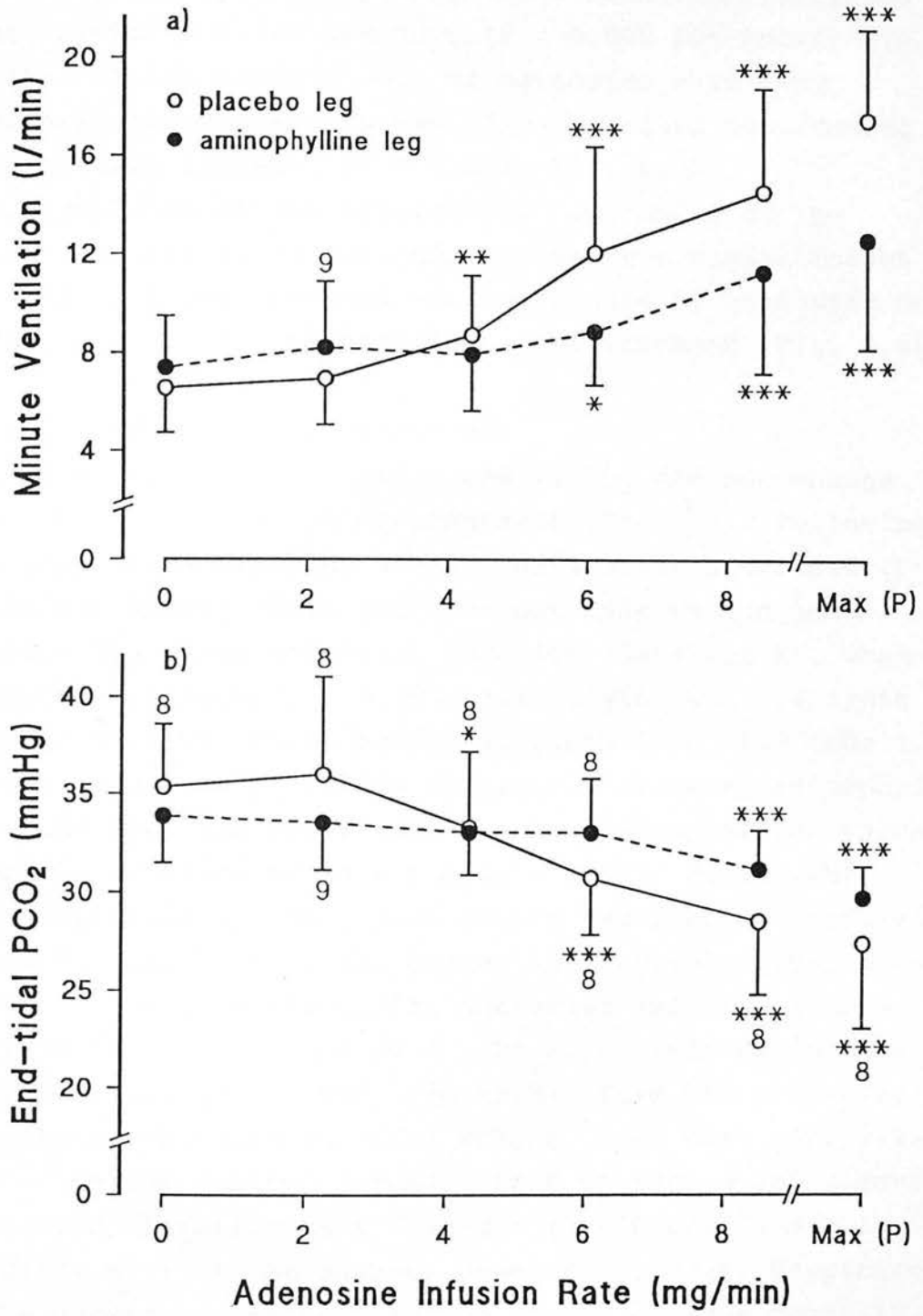

Fig. 5.5 - Effect of aminophylline on changes in minute ventilation and end-tidal PCO_2 during adenosine infusion. Abbreviations as in Fig. 5.3. *P < 0.05; **P < 0.01; ***P < 0.001 for comparisons with control (0 mg/min). n = 10 except where shown.

Following placebo pretreatment adenosine increased peak systolic blood pressure ($P < 0.001$ for ANOVA; Fig. 5.3b). At the maximum dose of adenosine mean peak systolic blood pressure was 16 mmHg higher than during the control infusion ($P < 0.002$; Fig. 5.3b). Aminophylline caused significant flattening of the dose-response curve for this effect ($P < 0.01$). Trough diastolic blood pressure was unaffected by adenosine both after placebo and aminophylline pretreatment (Fig. 5.3b).

5.3.4 - CHANGES IN RESPIRATION

Baseline minute ventilation and $PETCO_2$ did not change following the placebo pretreatment (Fig.5.4). Following aminophylline baseline minute ventilation increased ($P < 0.01$ for ANOVA). This increase was most marked just before the first adenosine infusion (Baseline 1), when minute ventilation, 7.6 (SD: 3.1) l/min, was 1.4 l/min higher than the value before aminophylline, 6.3 (SD: 2.1) l/min (Fig. 5.4a). $PETCO_2$ decreased following aminophylline and remained lower than the pre-aminophylline value for the duration of that leg ($P < 0.001$; Fig. 5.4b).

Following placebo, peak minute ventilation increased from 6.6 (SD: 1.8) l/min during the control infusion to 14.6 (SD: 4.3) l/min during adenosine infusion at 8.5 mg/min ($P < 0.001$) and 17.6 (SD: 3.7) l/min during the maximum dose ($P < 0.001$; Fig. 5.5). This was primarily due to an increase in tidal volume, from 0.45 (SD: 0.24) l during the control infusion to 0.85 (SD: 0.18) l during adenosine infusion at 8.5 mg/min ($P < 0.001$) and 1.10 (SD: 0.35) l at the maximum dose ($P < 0.001$). Respiratory rate during adenosine infusion at the maximum dose, 17.2 breaths/min, was not significantly different from the control value, 16.0 breaths/min. Trough $PETCO_2$ decreased during adenosine infusion from 35.3 (SD: 3.2) mmHg during the control infusion to 28.6 (SD: 3.8) mmHg during adenosine infusion at 8.5 mg/min ($P < 0.001$) and 27.4 (SD: 4.4) mmHg at the maximum dose ($P < 0.001$; Fig. 5.5).

Aminophylline significantly flattened the

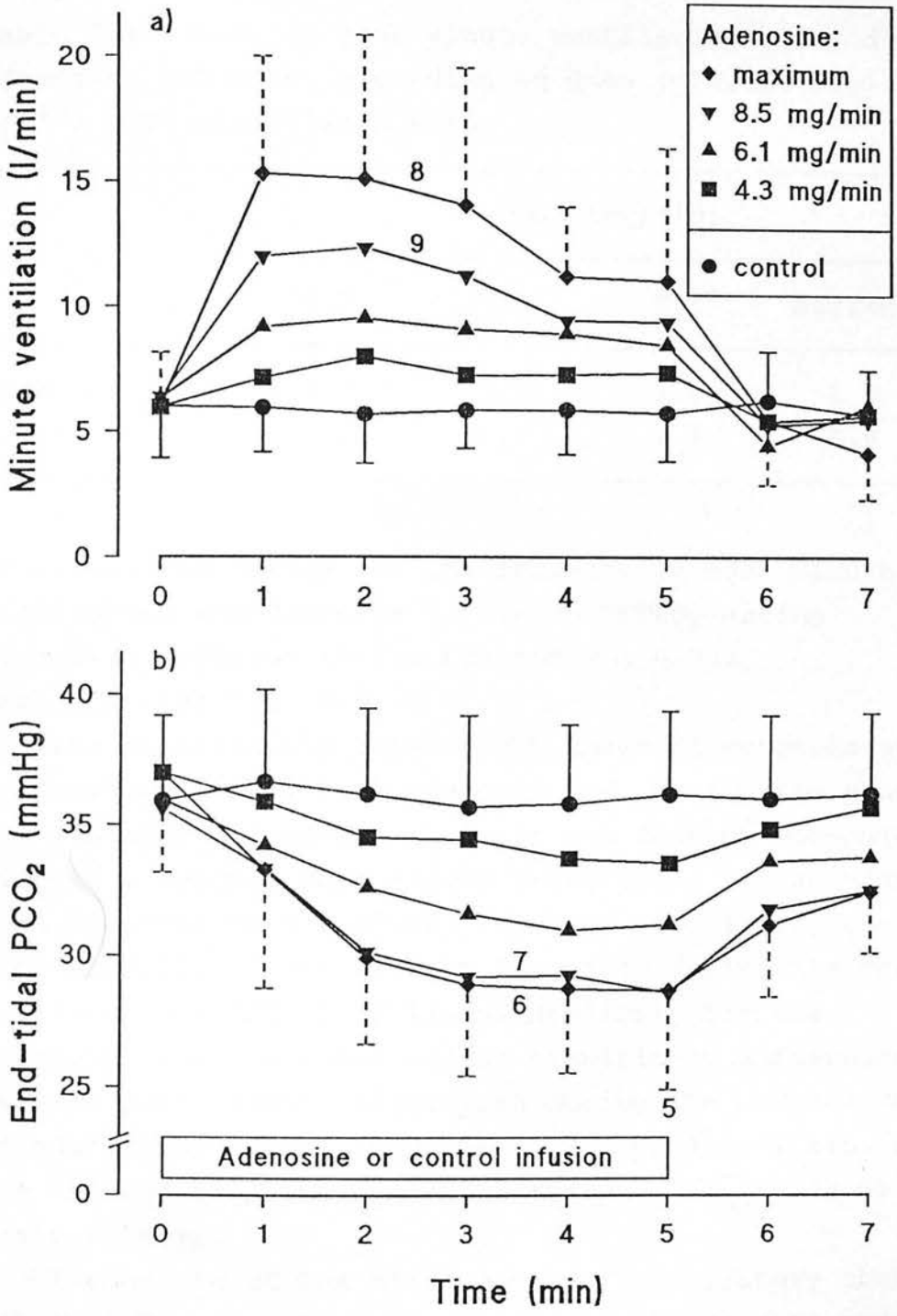

Fig. 5.6 - Time course of changes in minute ventilation and end-tidal PCO_2 during adenosine or control infusion following placebo pretreatment. n = 10 for minute ventilation and n = 8 for PCO_2 except where shown. Adenosine infusion at 2.3 mg/min omitted for clarity.

Table 5.1 - Time to peak minute ventilation (min) during adenosine infusion, according to dose (placebo leg). *n = 9; **n = 8: otherwise n = 10.

	Dose (mg/min)			
	4.3	6.1	8.5*	Maximum**
Mean	3.0	2.6	2.0	1.9
SD	1.6	1.7	1.1	0.9

dose-response curves for the increase in peak minute ventilation and decrease in trough $PETCO_2$ during adenosine infusion ($P < 0.001$ and $P < 0.001$, respectively; Fig. 5.5).

The relationship between the onset of symptoms and of respiratory stimulation was examined. During the placebo leg 8 subjects received at least one dose of adenosine without symptoms. Peak minute ventilation at the highest such dose for each subject, 7.4 (SD: 2.3) l/min, was not significantly different from the value during the control infusion, 6.6 (SD: 2.1) l/min. Similarly for the aminophylline leg there was no significant difference between peak minute ventilation during the maximum dose of adenosine without symptoms, 9.2 (SD: 3.1) l/min, and the value during the control infusion, 7.4 (SD: 2.2) l/min (n = 9).

Inspection of the time course of ventilatory changes within infusions at each dose showed that in the majority of subjects minute ventilation increased to a peak and then decreased. This effect was most marked at the higher doses. There was some inter-subject variability in the time to peak response and the peaks tended to be earlier with higher doses (Table 5.1).

For the group mean minute ventilation reached a peak 1 or 2 min after the onset of infusion at each dose (Fig.

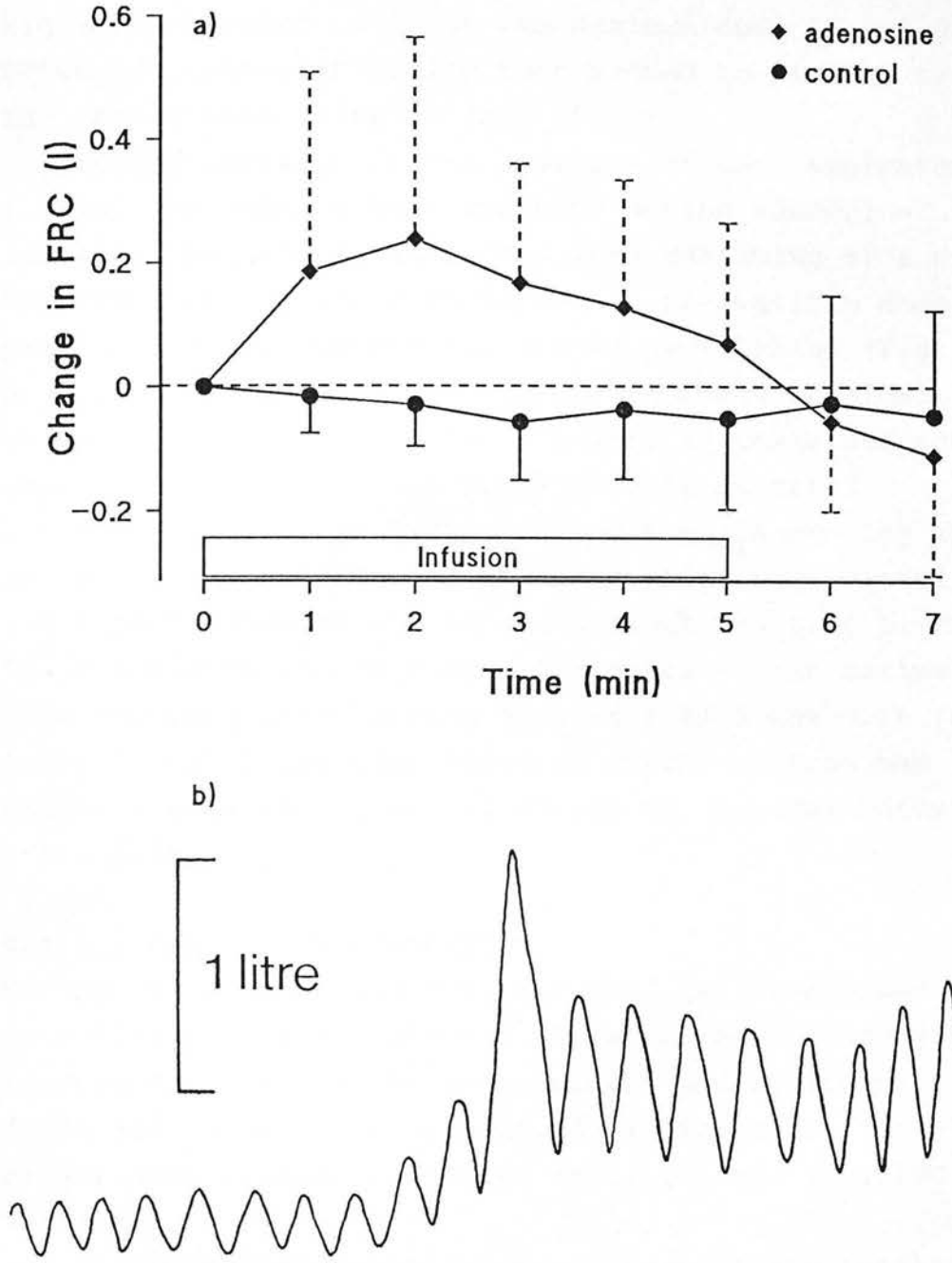

Fig. 5.7 - Changes in functional residual capacity (FRC) during adenosine infusion following placebo pretreatment. a) Changes during adenosine infusion at the maximum dose compared with changes during the control infusion. b) Respiratory trace from a single subject showing an increase in FRC occurring simultaneously with an increase in tidal volume.

5.6a). Comparison of values at 1 and 5 minutes showed a significant change only at the maximum dose ($P < 0.05$). $PETCO_2$ decreased initially then seemed to plateau towards the end of each infusion (Fig. 5.6b).

Upward movement of the baseline of the respiratory tracing was seen in most subjects during adenosine infusion, suggesting that they were breathing at a higher FRC. This effect was most marked at the maximum dose and parallelled the increase in minute ventilation (Fig. 5.7a). In a few subjects a marked increase in tidal volume developed over a few breaths. In these subjects a shift in the respiratory trace clearly occurred simultaneously (Fig. 5.7b). During the placebo leg at the maximum dose of adenosine the peak shift was greater than 0.2 l in 7 subjects and in one subject was 0.96 l. For the 8 subjects who completed 5 minutes at the maximum dose the mean shift during minutes 1 to 5 was 0.16 (SD: 0.24) l, which was significantly different from the shift, - 0.04 (SD: 0.09) l, during the control infusion (Fig. 5.7a).

5.3.5 - CHANGES IN SPIROMETRY

During the placebo leg FEV_1 and $FEF_{25-75\%}$ decreased significantly ($P < 0.001$ for ANOVA for both) between the measurements before the pretreatment and following the first adenosine infusion (Fig. 5.8). There were no other significant changes following this. FVC and FEV_1/FVC were unchanged.

Following aminophylline there were slight increases in FEV_1 and FVC which did not reach statistical significance. FEV_1/FVC and $FEF_{25-75\%}$ were unchanged (Fig. 5.9).

In the subject with asthma adenosine infusion caused bronchoconstriction during the placebo leg but this was not seen with the same maximum dose of adenosine during the aminophylline leg (Fig. 5.10).

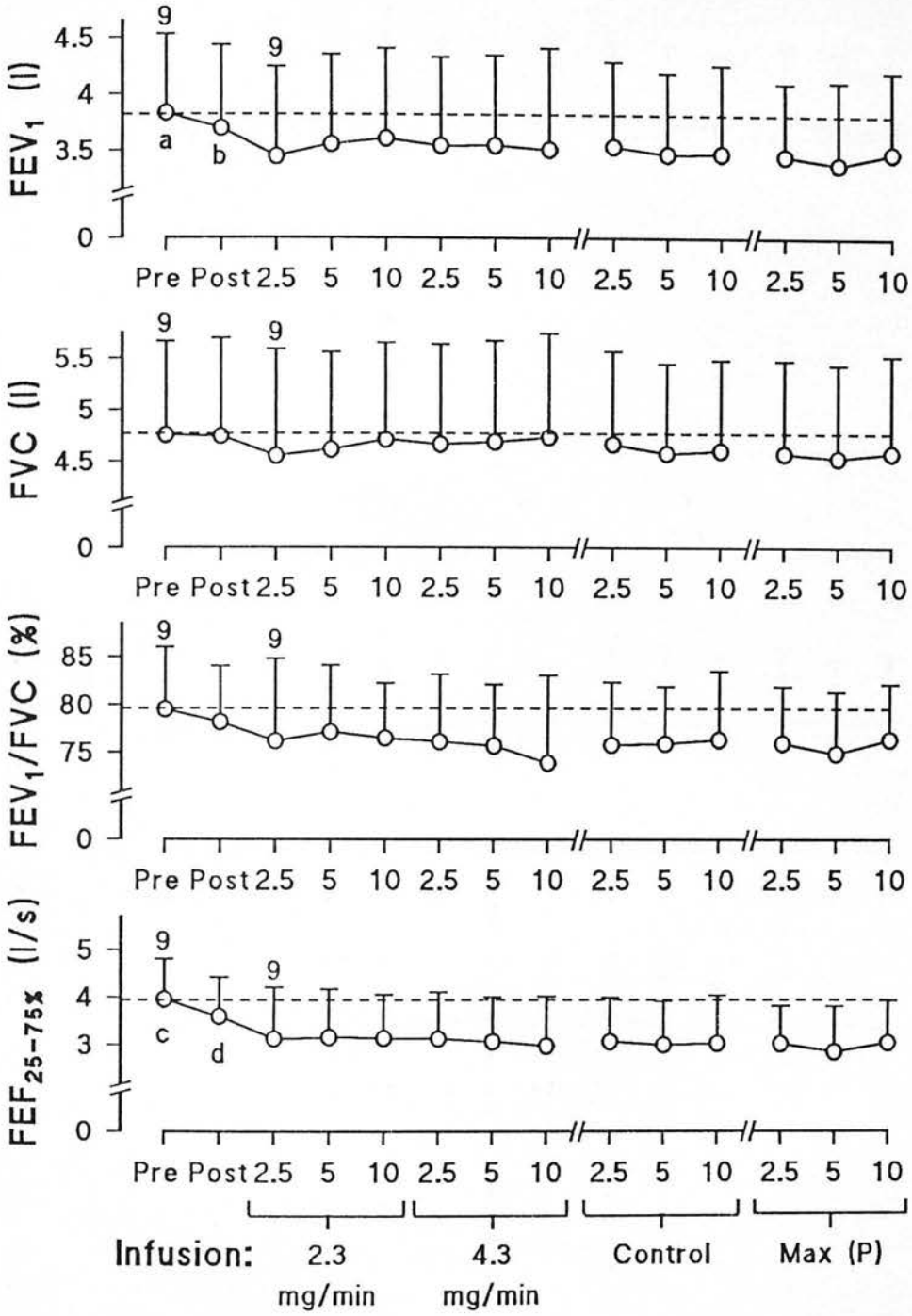

Fig. 5.8 - Changes in spirometric variables during the placebo leg. n = 10 except where shown.

Pre & Post, prior to and following the placebo pretreatment respectively; Max (P), the maximum dose received by each subject. Times 2.5, 5, 10 represent 2.5, 5 and 10 minutes following an infusion. a, greater than all except "Post" value (P: < 0.001 to < 0.05); b, greater than last 5 values (P: < 0.05 to < 0.001); c, greater than all except "Post" value (P < 0.001); d, greater than 5 values (all following 2.5 mg/min, 10 min following control and 5 min following maximum dose; P: < 0.05 to < 0.001).

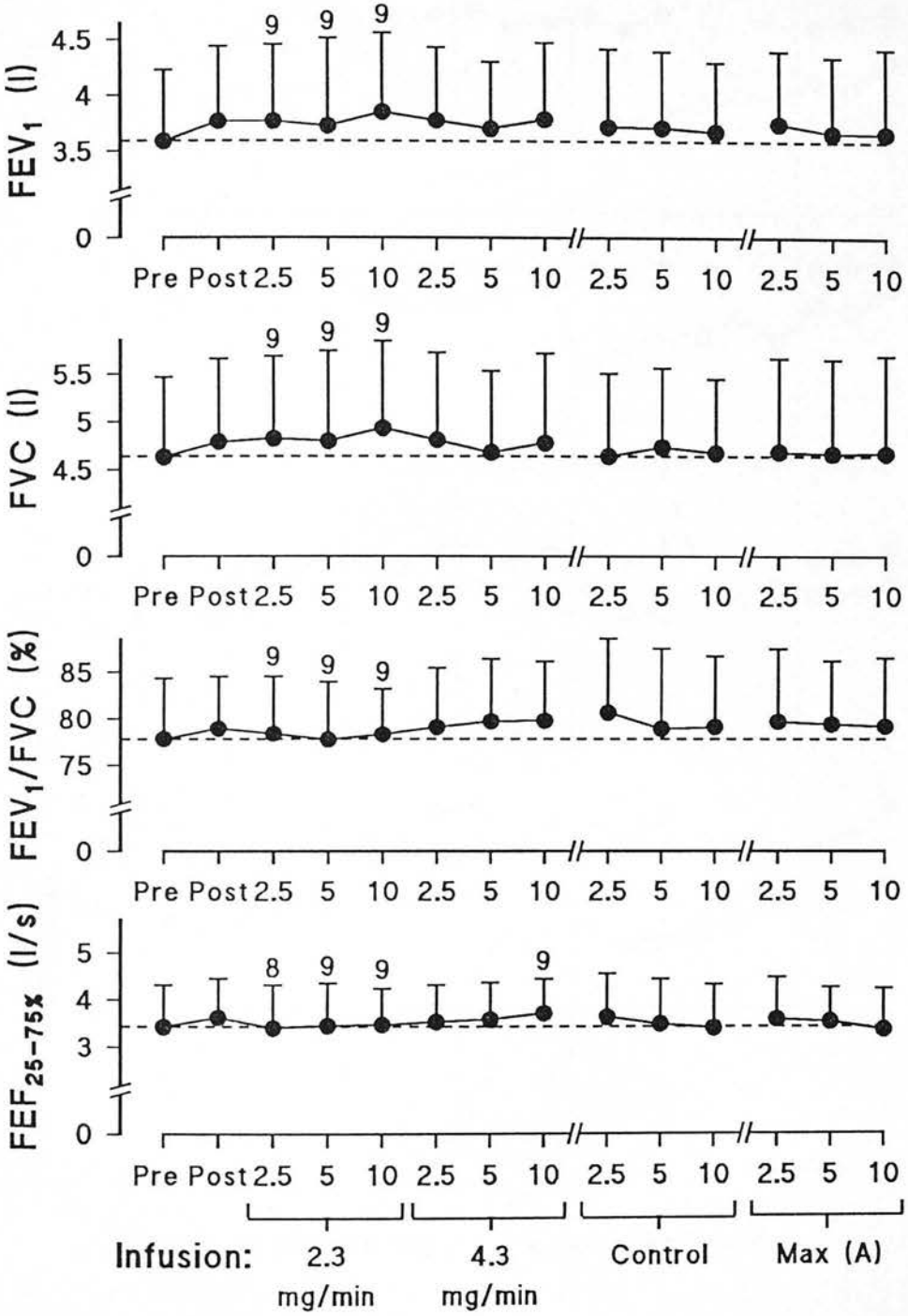

Fig. 5.9 - Changes in spirometric variables during the aminophylline leg. n = 10 except where shown.
Max (A), the maximum dose received by each subject during the aminophylline leg. Other abbreviations as in Fig. 5.8.

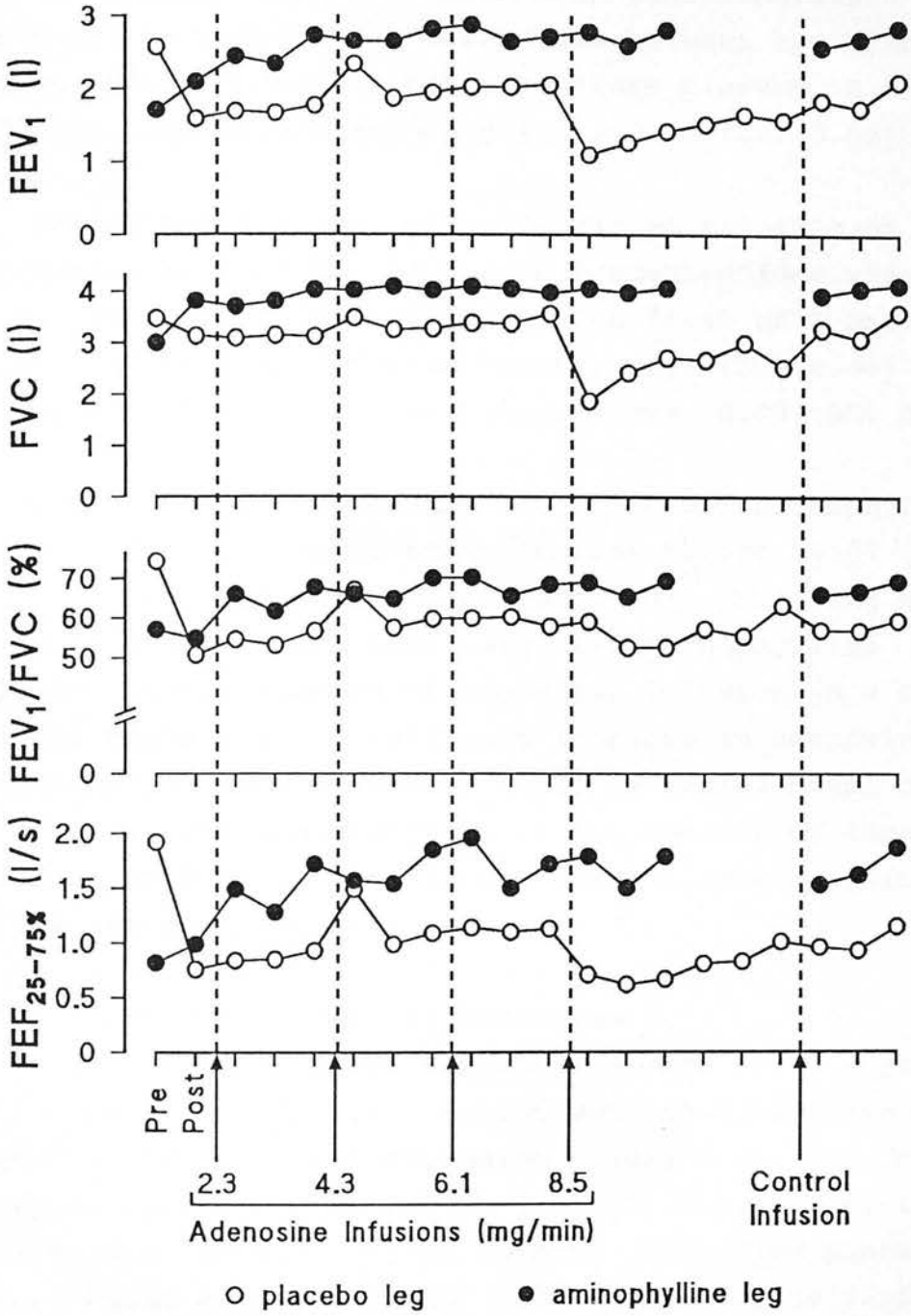

Fig. 5.10 - Changes in spirometric variables in a patient with asthma. Spirometry was performed 2.5, 5 and 10 min after each infusion and also at 15, 20 and 25 min following adenosine infusion at 8.5 mg/min during the placebo leg, because of evidence of bronchoconstriction following that infusion. Abbreviations as in Fig. 5.8.

5.3.6 - CHANGES IN PLASMA ADENOSINE CONCENTRATION

There was no significant difference between the venous plasma adenosine concentrations before placebo, 0.07 (SD: 0.04) μM, and before aminophylline, 0.08 (SD: 0.02) μM (n = 6).

During the placebo leg there was no significant difference between the adenosine concentrations measured in the absence of adenosine infusion (mean of 3 baseline values and control infusion value), 0.07 (SD: 0.04) μM, and during infusion at the maximum dose, 0.09 (SD: 0.07) μM (n = 5).

During the aminophylline leg there was no significant difference in adenosine concentration before, 0.07 (SD: 0.03) μM, and following, 0.08 (SD: 0.03) μM (using means of up to 3 values for each subject), aminophylline infusion in the absence of adenosine infusion (n = 6). However there was a significant increase in adenosine concentration from 0.08 (SD: 0.02) μM (using means of up to 4 values for each subject) in the absence of adenosine infusion to 0.10 (SD: 0.03) μM during adenosine infusion at the maximum dose ($P < 0.02$, n = 6).

5.3.7 - SYMPTOMS CAUSED BY ADENOSINE

Symptoms occurred in all subjects on both study days. Following aminophylline symptoms were fewer despite the higher maximum dose of adenosine (Table 5.2). The onset of first symptoms occurred at 4.9 (SD: 1.9, range: 2.3 to 6.1) mg/min (70 (SD: 28) μg/kg/min) following placebo pretreatment and at 8.0 (SD: 4.3, range: 2.3 to 16.8) mg/min (112 (SD: 58) μg/kg/min) following aminophylline.

5.4 - DISCUSSION

This study examined the effects of aminophylline on the responses to adenosine infusion in normal volunteers. As in the study described in Chapter 3, adenosine caused significant increases in minute ventilation , tidal volume, heart rate and systolic blood pressure and a significant decrease in end-tidal PCO_2. However in this

Table 5.2 - Symptoms during adenosine infusion.

Symptom	Number of subjects with symptom	
	Placebo leg	Aminophylline leg
Epigastric discomfort	8	4
Dyspnoea	7	5
Flushing (subjective)	6*	4
Chest discomfort	4	5
Throat, jaw discomfort	4	1
Paraesthesiae	4	1
Shoulder pain	2	0
Nausea	2	1
Headache	1	2
Palpitations	2	1
Dizziness	1	1
Eye irritation	0	1
Altered taste	0	1
Sweating	1	0
Exhaustion	0	1
Wheeze	1	0
Total	43	28

* 3 further subjects were visibly flushed during the placebo leg

study diastolic blood pressure was unchanged.

5.4.1 - AMINOPHYLLINE ANTAGONISM OF ADENOSINE EFFECTS

A single infusion of aminophylline of 6 μg/kg/min over 10 min was sufficient to achieve mean serum theophylline concentrations of approximately 8 to 10 mg/l for the duration of the relevant leg. There were few side-effects attributable to aminophylline, which caused significant

antagonism of the effects of adenosine on ventilation and $PETCO_2$, heart rate and systolic blood pressure. Furthermore following aminophylline symptoms due to adenosine began at a higher mean dose and were fewer despite the higher maximum infusion rate of adenosine given.

Since *in vitro* studies have shown that theophylline at a concentration of approximately 10 mg/l causes significant antagonism of adenosine at cell-surface receptors (see Chapter 1) the findings of this study suggest that the direct effects of adenosine may be mediated by cell-surface receptors. As discussed in Chapter 1 antagonism by theophylline at such concentrations is unlikely to be due to phosphodiesterase inhibition.

Other workers have examined the effects of methylxanthines on the cardiorespiratory responses to adenosine in animals and man. As discussed in Chapter 1 methylxanthines have been shown to antagonise the respiratory depressant effects of adenosine and its analogues within the central nervous system (eg. Kattwinkel & Darnall, 1982; Wessberg *et al.*, 1985). Antagonism of the ventilatory stimulation caused by intracarotid injections of an adenosine analogue in anaesthetised rats was reported by Monteiro and Ribeiro in 1987. In anaesthetised dogs aminophylline antagonises the coronary and systemic haemodynamic effects of adenosine (Afonso, 1970; Afonso & O'Brien 1970).

In man, Routledge and Watt (1986) reported preliminary findings that aminophylline antagonized the adenosine-induced bradycardia but did not significantly affect the subsequent tachycardia or respiratory stimulation produced by adenosine boluses. However, consistent with the present results, Jonzon *et al.* (1989) reported that theophylline does antagonise the ventilatory effect of bolus injections of adenosine. Furthermore Smits *et al.* (1987) reported that caffeine significantly reduced the adenosine-induced changes in minute ventilation, tidal volume, respiratory frequency,

venous PCO_2 and pH. Similarly Maxwell *et al.* (1987) observed antagonism of the effects of adenosine on ventilation and estimated arterial PCO_2 by theophylline. They also showed that theophylline reduced symptoms caused by adenosine allowing higher infusion rates to be given. Both Smits *et al.* (1987) and Maxwell *et al.* (1987) observed no reduction of adenosine effects by enprofylline a xanthine which does not antagonize adenosine *in vitro* (Persson *et al.*, 1982).

Further confirmation that the cardiorespiratory effects of adenosine are mediated by cell-surface receptorscomes from reports that such effects are potentiated by dipyridamole, which increases extracellular concentrations of adenosine by inhibiting its transport into cells. Watt *et al.* (1986a) observed potentiation of the effects of adenosine on the sinus node by dipyridamole. Watt and Routledge (1987d) reported that dipyridamole potentiated the initial bradycardia and the ventilatory stimulation caused by intravenous bolus injections of adenosine. Biaggioni *et al.* (1986) showed that after dipyridamole 4-fold lower infusion rates of adenosine were required to produce a given increase in heart rate and systolic blood pressure and fall in diastolic blood pressure.

5.4.2 - EFFECTS OF AMINOPHYLLINE *PER SE* ON RESPIRATION

In this study aminophylline caused an increase in resting minute ventilation and decrease in $PETCO_2$. It has long been known that methylxanthines can increase minute ventilation in man (Edsall & Means, 1914) and such compounds have been used to treat Cheynes Stokes respiration (Vogl, 1927) and neonatal apnoea (Kuzemko & Paala, 1973). Morice *et al.* (1986) reported that aminophylline potentiates the ventilatory response to hypercapnia although some earlier workers had been unable to show this (Stroud *et al.*, 1955; Lakshminarayan *et al.*, 1978). Lakshminarayan *et al.* (1978) and Sanders *et al.* (1980) showed that aminophylline potentiates the

ventilatory response to hypoxia. Aubier (1981) reported that theophylline increased contractility and reduced fatigue in the diaphragm of normal subjects and suggested that increased diaphragmatic efficiency caused the theophylline-induced increase in ventilation which they observed in anaesthetised, spontaneously breathing dogs (Aubier et al., 1983). However not all workers have been able to demonstrate effects of theophylline on diaphragmatic contractility in normal subjects (Moxham et al., 1985).

Since adenosine and its analogues depress ventilation by an action within the central nervous system (see Chapter 1) and since such effects are antagonised by theophylline it has been proposed that methylxanthines may stimulate respiration by antagonism of the action of endogenous adenosine (Millhorn et al., 1984). Therefore the intriguing possibility exists that in the present study aminophylline influenced the adenosine-ventilation dose response curve by antagonism of two opposing effects of adenosine, one mediated peripherally the other exerted directly within the central nervous system.

5.4.3 - CHANGES IN SPIROMETRY

The cause of the initial fall in FEV_1 and $FEF_{25-75\%}$ during the placebo leg is uncertain. It seems unlikely that this was due to an effect of the first, and lowest, dose of adenosine since subsequent infusions at higher rates caused no further change. Since these measurements were made semi-recumbent it is possible that a change occurred in subjects' posture early in the study although a foot board was used to prevent subjects slipping down the bed. If fatigue or bronchoconstriction, due to subjects performing multiple forced expirations, were the cause a progressive fall during the study would have been expected. Aminophylline caused an increase in FEV_1 suggesting that it may have caused bronchodilation. It therefore offset or possibly antagonised the mechanism of the fall noted during the placebo leg. Persson (1980)

showed *in vitro* that theophylline causes relaxation of airways from non-obstructed patients and *in vivo* theophylline has been shown to increase airway conductance in normal subjects by some, but not all, workers (Mackay *et al.*, 1983; Estenne *et al.*, 1980), a response possibly in part due to stimulation of catecholamine release (Higbee *et al.*, 1982).

The lack of an effect of adenosine on the indices of airway calibre suggests that bronchoconstriction was not responsible for the changes in breathing. Fuller *et al.* (1987) also assessed airway calibre by a forced expiratory flow volume curve. They found no significant change in air flow at 30% of a reference vital capacity during adenosine infusion up to 100 μg/kg/min. Larsson and Sollevi (1988) found no change in expiratory flow volume curves, specific airway conductance (a more sensitive measure of airway calibre), or bronchial reactivity during adenosine infusion in asthmatic subjects. However their maximum infusion rate was only 50 μg/kg/min.

Although the bronchoconstriction observed in the single patient with asthma in this study might have been caused by the adenosine-induced hyperventilation (Deal *et al.*, 1979) it might have been due to a direct bronchoconstrictor effect of adenosine, as has been observed with the inhaled compound (see Chapter 1). Intravenous adenosine has been reported to cause bronchoconstriction in rats (Pauwels & Van Der Straeten, 1983) and a case of bolus injections of adenosine exacerbating asthma in man has been reported (Taviot *et al.*, 1986). These observations suggest that caution should be used in administering adenosine to patients with asthma.

5.4.4 - TIME COURSE OF ADENOSINE-INDUCED CHANGES

Analysis of the time course of changes in ventilation confirms that, although there is some inter-subject variability in the time to peak response, it is

reasonable to use mean values 1 or 2 minutes after a change in infusion rate to construct dose-response curves as was done in Chapter 3. However a better approach may be to use areas under the curve or, as was done here, the peak response, provided comparison is made with the equivalent measurement during the control infusion.

Antagonistic effects on the chemoreceptors of the reduced arterial PCO_2 will have contributed to the reduction of minute ventilation towards the end of infusions at the higher doses. Whether this was the only mechanism is uncertain. However there was no evidence of a return towards normal of end-tidal PCO_2 which might have been seen had tachyphylaxis occurred.

The significance of the shift upwards in the baseline of the respiratory trace during adenosine infusion and the reasons why this was probably due to an increase in FRC will be discussed in Chapter 8.

5.4.5 - CHANGES IN PLASMA ADENOSINE CONCENTRATION

These results show that aminophylline has no effect on basal peripheral venous plasma concentrations of adenosine. That the concentration of adenosine was only increased during infusion at the higher maximum dose possible after aminophylline is consistent with *in vitro* studies which suggest that the half-life of adenosine in human blood is only a few seconds (Klabunde 1983; Möser *et al.*, 1989). Furthermore *in vivo* vascular epithelium probably also avidly takes up adenosine. The peripheral venous concentration is therefore not a useful index of concentrations pertaining much more proximally in the circulation during intravenous infusion of adenosine.

CHAPTER 6 - CHANGES IN ARTERIAL PLASMA ADENOSINE CONCENTRATION DURING ADENOSINE-INDUCED RESPIRATORY STIMULATION

6.1 - INTRODUCTION

Watt & Routledge (1985) proposed that adenosine may be a physiological mediator or modulator of the ventilatory response to hypoxia in the carotid bodies. This hypothesis has gained support from the observation that intravenous infusion of adenosine potentiates the ventilatory response to hypoxia (Maxwell et al., 1986).

However whether the effects of adenosine occur at concentrations likely to be achieved in vivo is unknown and was therefore investigated in this study. Since adenosine has a very short half-life in blood (Möser et al., 1989) and since the recirculating plasma venous adenosine concentration changes little during exogenous infusion, as discussed in Chapter 5, in the present study blood was sampled from a location as close as practicable to the putative site of action in the carotid bodies.

6.2 - METHODS

6.2.1 - SUBJECTS

The subjects were 7 patients (5 male) aged 46 to 65 years (mean 57) scheduled to undergo investigation of chest pain by cardiac catheterisation (Table 6.1). This afforded an opportunity to sample blood from a site close to the carotid circulation. Six of the patients had coronary artery disease. None had clinical evidence of heart failure or a history of asthma or other chronic chest disease. All continued their usual medications (Table 6.1) and received diazepam 10 mg orally 1 hour prior to catheterisation.

Prior to the study, which was performed before angiography, a pigtail catheter (8F) was inserted via a femoral artery sheath (8F) and positioned with its tip in the aorta just distal to the origin of the left common

Table 6.1 - Patient characteristics.

Patient	Age (years)	Sex	Height (m)	Weight (kg)	Treatment	Diagnosis
1	55	M	1.78	92	B N	3-VD
2	58	M	1.71	80	B N	2-VD
3	63	F	1.59	63	BCNA	2-VD
4	46	M	1.75	69	B A	1-VD
5	52	M	1.80	96	N	2-VD
6	65	M	1.72	78	B N	2-VD
7	60	F	1.50	49	BC	No CAD
Mean	57	-	1.69	75	-	-
SD	7	-	0.11	16	-	-

M, male; F, female; CAD, coronary artery disease; 1-VD, 1 vessel coronary artery disease; 2-VD, 2 vessel disease; 3-VD, 3 vessel disease; B, ß-adrenoceptor antagonist; C, calcium antagonist; N, long-acting nitrate; A, aspirin.

carotid artery. A femoral venous sheath (7F) was also inserted and following an equilibration period the study was begun.

6.2.2 - INFUSION PROTOCOL

Following baseline measurements and samples adenosine was infused via the femoral venous sheath using a protocol similar to that employed in the study described in Chapter 3. The initial infusion rate, 4.3 mg/min, was followed by stepwise increments every 2 min (to 6.1, 8.5, 11.9 and 16.8 mg/min) until limited by symptoms. This duration of the infusion stages was considered sufficient because of the findings discussed in Chapter 5 that mean minute ventilation reaches its maximum value 1 to 2 minutes following the commencement of infusion at a given dose and that there is no significant difference between

values at 1 and 2 minutes. Symptoms were recorded at the end of each stage.

6.2.3 - MEASUREMENTS AND SAMPLES

Heart rate, blood pressure and respiration were monitored continuously (except for a few seconds during arterial blood sampling in the case of blood pressure) and recorded prior to the infusion (baseline), during the last 45 s of each infusion stage and 2 min after stopping the infusion.

An electrocardiogram (modified standard lead 2) was used to determine heart rate. Blood pressure was measured via the arterial catheter using a Bell and Howell strain gauge transducer, a Cambridge amplifier and a Mingograph multichannel recorder. Respiration was recorded as described in Chapter 2 using a respiratory inductance plethysmograph connected to a chart recorder (Rikadenki).

Blood was sampled from the aortic arch via the catheter at baseline, 15 s before the end of each stage and 2 min post-infusion for measurement of plasma adenosine concentration. The sampling technique and assay are described in Chapter 2.

6.2.4 - STATISTICAL ANALYSIS

Baseline data (log-transformed in the case of minute ventilation, tidal volume and plasma adenosine concentration) were compared with values obtained during the last complete infusion stage and 2 min following the infusion using repeated measures analysis of variance and the Student-Newman-Keuls test.

6.3 - RESULTS

The maximum dose of adenosine administered was limited in all patients by symptoms and during the final complete stage, ranged from 6.1 to 16.8 mg/min (mean 10.8 mg/min) (88 to 182 μg/kg/min, mean: 130 μg/kg/min). In 4 patients the final infusion stage was stopped prematurely at the patients request. In these cases, data from the last

complete stage were used in the analysis.

6.3.1 - CHANGES IN PLASMA ADENOSINE CONCENTRATION

The mean plasma adenosine concentration increased from 0.07 (range: 0.04 to 0.12) μM at baseline to 1.20 (range: 0.42 to 2.04) μM during the final complete stage ($P < 0.001$) (Fig. 6.1). There was a threshold infusion rate, which varied between patients, below which aortic plasma adenosine concentration did not change. The adenosine concentration 2 min post infusion was 0.07 (range: 0.03 to 0.10, $n = 6$) μM which was not different from the baseline value (Fig. 7.1).

6.3.2 - CHANGES IN RESPIRATION

Minute ventilation increased from 5.5 (SD: 1.9) l/min at baseline to 10.9 (SD: 3.3) l/min at the maximum dose of adenosine ($P < 0.001$) (Fig 2). This was almost entirely due to a 101% increase in tidal volume from 0.33 (SD: 0.12) l to 0.66 (SD: 0.25) l ($P < 0.005$). There was no significant change in respiratory frequency which was 17 (SD: 3) breaths/min at baseline and 18 (SD: 4) breaths/min at the maximum dose of adenosine. Two minutes following the infusion minute ventilation was 5.0 (SD: 1.6) l/min which was not different from the baseline value, but tidal volume, although less than during the infusion ($P < 0.05$), remained 74% higher than the baseline value ($P < 0.05$), and respiratory rate fell to 12 (SD: 5) breaths/min ($P < 0.025$) indicating a change in breathing pattern.

6.3.3 - CHANGES IN HEART RATE AND BLOOD PRESSURE

Heart rate increased from 61 (SD: 12) beats/min at baseline to 72 (SD: 14) beats/min at the maximum dose ($P < 0.01$) and following the infusion fell to 67 (SD: 15) beats/min which was not significantly different from the baseline value. There were no significant changes in systolic, diastolic or mean blood pressure (138 (SD: 20), 73 (SD: 17) and 96 (SD: 15) mmHg respectively at baseline

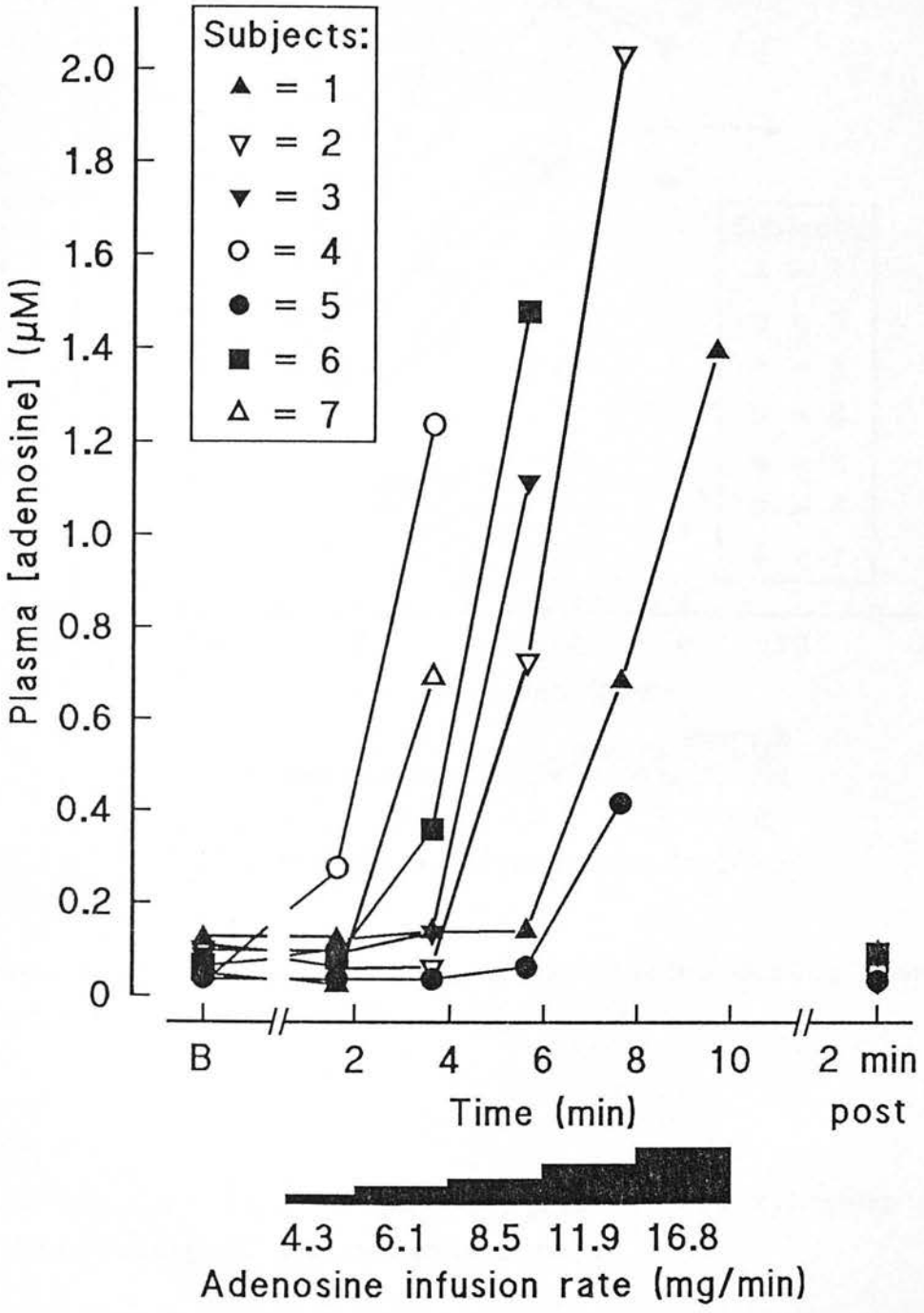

Fig. 6.1 - Changes in arterial plasma adenosine concentration during adenosine infusion. B, baseline.

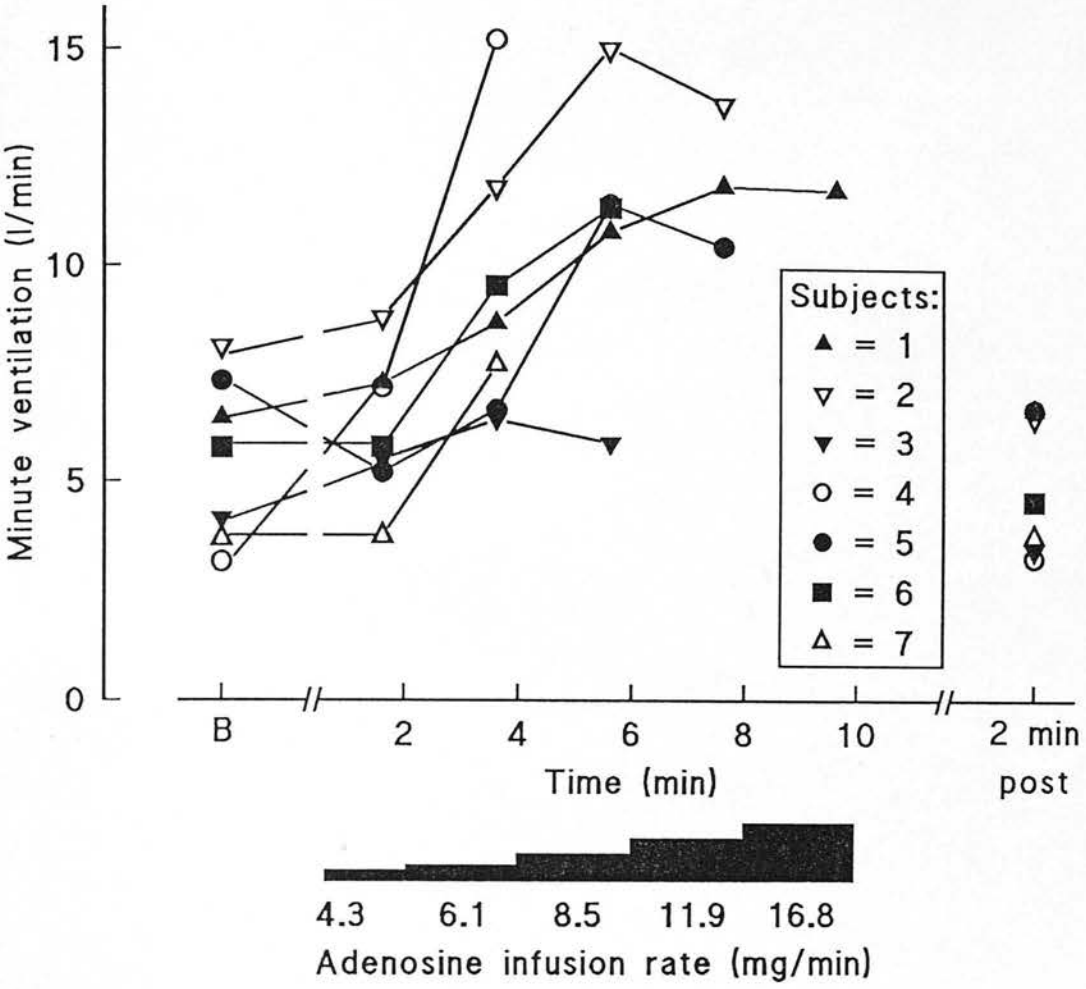

Fig. 6.2 - Changes in minute ventilation during adenosine infusion. B, baseline.

and 135 (SD: 21), 71 (SD: 20) and 98 (SD: 21) mmHg respectively at the maximum dose).

6.3.4 - INDIVIDUAL RESPONSES

In 3 patients (numbers 1,2,3) an initial increase in minute ventilation (of 69% by the end of the third infusion stage, 46% during the second stage and 33% during the first stage respectively) was accompanied by little change in the plasma adenosine concentration (0.12 to 0.14 μM, 0.10 to 0.06 μM and 0.09 to 0.09 μM respectively) or heart rate (62 to 62, 56 to 56 and 70 to

64 beats/min respectively) although all subsequently showed increases in heart rate and adenosine concentration. In the remaining patients minute ventilation, heart rate and adenosine concentration increased in concert.

6.3.5 - SYMPTOMS CAUSED BY ADENOSINE

Symptoms reported were similar to those experienced in the other studies described in this thesis and included dyspnoea in 5 patients, flushing, chest discomfort and throat discomfort each in 3 patients, headache and limb paraesthesiae each in 2 patients and drowsiness and altered taste each in 1 patient. All symptoms resolved within 1 to 2 min of discontinuing the infusion.

6.4 - DISCUSSION

In this study the relationship between the adenosine concentration in arterial plasma from the distal aortic arch and minute ventilation during intravenous infusion of adenosine were examined. An increase in mean adenosine concentration from 0.07 to 1.20 μM was associated with a doubling in minute ventilation, a slight increase in heart rate and symptoms similar to those reported previously, but no significant change in arterial blood pressure.

6.4.1 - CHANGES IN RESPIRATION

The present unexpected finding that ventilation increased in 3 subjects in the absence of a detectable increase in adenosine concentration in the aorta might have been due to spontaneous variation in respiration. Alternatively it suggests that adenosine might stimulate respiration by an intra-pulmonary effect. Blood cells and pulmonary endothelial cells avidly take up adenosine (Hellewell & Pearson, 1983; Catravas, 1984; Das & Steinberg, 1988) so it would be possible for increased concentrations to pertain in the pulmonary circulation while little change is detected more distally. Sylvén *et al*. (1987) reported

that the onset of respiratory stimulation by bolus injections preceded the development of atrioventricular block consistent with an action of adenosine in the lung, although their observations contrasted with the earlier findings of Watt and Routledge (1985) that the longest R-R interval on the ECG, reflecting both sinus slowing and atrioventricular block (Watt & Routledge, 1986b), occurred up to 2 s before the onset of the increase in respiration. Preliminary observations in the anaesthetised cat showed that adenosine stimulates intra-pulmonary vagal afferents (Cherniack et al., 1987) and increases respiration by an action unaffected by sino-aortic denervation (Runold et al., 1987). This however contrasts with the findings that respiratory stimulation by adenosine in the rabbit and rat is abolished by section of the nerve supply to the carotid body (Buss et al., 1986; Monteiro & Ribeiro, 1987).

Symptoms or psychogenic factors might have contributed to the respiratory changes in the present study. That ventilation was increased largely by an increase in tidal volume is consistent with a chemoreceptor-mediated effect (Jennett et al., 1974; Newsom Davis and Stagg, 1974). However anxiety or unpleasant sensations have been reported to affect breathing in different ways (Cunningham & Gardiner, 1972; Hugelin, 1986) so it is difficult to predict the possible effects of combinations of such stimuli. Nevertheless as described in Chapter 4 adenosine infusion into the descending thoracic aorta was not associated with increased ventilation although the majority of subjects experienced chest or epigastric discomfort. Furthermore, as discussed in Chapter 3, while a single-blind infusion of adenosine caused a degree of respiratory stimulation in normal volunteers similar to that observed in the present study, a single-blind infusion of inosine caused no significant change, suggesting that the response to adenosine was not due to subjects' expectations. For these reasons a placebo was not used in the present

study.

Other factors might have affected the ventilatory responses observed. Changes in blood gas tensions will have antagonised the increases in ventilation as discussed in Chapter 5. Other factors which might have affected the responses to adenosine, such as changes in cardiac output and left ventricular filling pressure and the other medications patients received, will be discussed in Chapter 7.

6.4.2 - CHANGES IN PLASMA ADENOSINE CONCENTRATION

Sampling of blood through a catheter for measurement of adenosine concentration poses particular problems as discussed in Chapter 2. Nevertheless the measured values during adenosine infusion are likely to have been sufficiently good estimates of the true values to allow an answer to be given to the main question posed by this study, i.e.: "Are the concentrations of adenosine which cause respiratory stimulation likely to be achieved physiologically?"

There is considerable variation amongst previously reported values of plasma adenosine concentration in man, probably largely due to differences in sampling and assay techniques (Table 2.1). Recently reported values for resting levels of adenosine in man range from 0.02 μM (Mann et al., 1986) to 0.15 μM in venous blood (Sollevi, 1986), and 0.36 μM in arterial blood from patients with ischaemic heart disease (Sollevi, 1986), i.e. of a similar order of magnitude to the present data. It is of interest that Lönnroth et al. (1989) recently found, using a microdialysis technique, similar basal concentrations of adenosine (0.025 to 0.3 μM) in interstitial fluid of subcutaneous adipose tissue in humans.

During adenosine infusion radial artery plasma concentrations, which are probably lower than central arterial concentrations, have been measured by 2 groups. During hypotension induced by adenosine infusion at 140

(SD: 40) μg/kg/min into the superior vena cava in anaesthetised patients, Sollevi et al. (1984b) observed an increase in radial artery adenosine concentration from 0.15 (SD: 0.02) to 2.45 (SD: 0.65) μM. Their patients were pre-treated with dipyridamole, which probably increased plasma adenosine concentrations (Sollevi et al., 1984c). Biaggioni et al. (1986) observed an increase in radial artery adenosine concentration from 0.16 (SD: 0.07) to 0.41 (SD: 0.19) μM during peripheral intravenous infusion at 129 (SD: 8) μg/kg/min in conscious subjects. The increase in aortic plasma concentration observed in the present study, from 0.07 (SD: 0.03) to 1.2 (SD: 0.53) μM, during infusion at 130 (SD: 30) μg/kg/min is consistent with those results.

6.4.3 - ADENOSINE AND THE CONTROL OF BREATHING

Monteiro and Ribeiro (1987) showed that the adenosine-induced increase in ventilation in the rat is probably due to an effect at A_2 adenosine receptors, which generally mediate stimulation of adenylate cyclase (van Calker et al., 1979). The affinity of A_2 receptors shows wide variation with half-maximal effects reported at adenosine concentrations varying from less than 1 μM to over 10 μM depending on the tissue and species studied (Daly, 1983).

Due to rapid cellular uptake and metabolism of adenosine the maximum concentrations pertaining in the pulmonary circulation in this study may have been of the order of 10 to 20 μM, assuming a circulation time to the sampling point of 4 s (Treves et al., 1974) and a half-life of 1.5 s. Similarly the maximum concentrations in the carotid bodies may have been less than 1 μM. These results would therefore be consistent with an A_2-mediated intra-pulmonary effect of adenosine but do not exclude an effect in the carotid bodies.

Stimuli such as hypoxia which induce ATP degradation have been shown, predominantly in animals, to cause several-fold increases in adenosine concentration in a

number of tissues (Tables 6.2 and 6.3). In humans Sollevi (1986) reported plasma adenosine concentrations in umbilical arterial blood of hypoxic neonates ranging up to 2μM (mean 0.61 μM), i.e. several-fold higher than in normoxic adults. Concentrations in tissue interstitial fluid in that instance might have been even higher than plasma concentrations. These reports suggest that concentrations of adenosine similar to those observed in the present study may be achieved in vivo during states such as hypoxia.

In conclusion these results are consistent with the suggestion (Watt and Routledge, 1985) that adenosine released during hypoxia may mediate or modulate the resultant ventilatory stimulation. However, despite previous evidence suggesting that adenosine-induced respiratory stimulation is mediated by the carotid bodies, the finding that ventilation increased in 3 subjects without a detectable increase in plasma adenosine concentration suggests that an intra-pulmonary effect might also contribute.

Table 6.2 - Examples of increased tissue adenosine content in situations of increased ATP breakdown.

Species	Tissue	Stimulus	Increase in adenosine content	Reference
Rat	Brain	Hypoxia:		
		FiO2 10.7%	x3	
		FiO2 5.5%	x7	Rubio et al.,1975
Rat	Brain	Ischaemia:		
		5 s	x2	
		60 s	x6	Winn et al., 1979
Dog	Heart:	Ischaemia:		
	ventricle	15 s	x6	Olsson, 1970
Man	Heart:	Cardioplegia:		
	ventricle	15°C for 10 min	x4	Sollevi, 1986
Dog	Hindlimb:	Ischaemic		
	muscle	contraction:		
		5 min	x2	Dobson et al., 1971
Dog	Lung	Hypoxia:		
		95% N2 and		
		5% CO2 for		
		3 min	x9	Mentzer et al., 1975
Rat	Kidney	Ischaemia:		
		30 s	x3	
		60 s	x6	Osswald et al., 1977

Table 6.3 - Examples of increased adenosine concentrations in situations of increased ATP breakdown.

Species	Fluid	Stimulus	Change in adenosine concentration* (μM)	Reference
Piglet	Brain: interstitial fluid	Hypoxia: P_{O2} 20mmHG for 10 min		
	a) Frontal cortex		0.68 to 1.60	
	b) Thalamus		1.03 to 2.60	Park et al., 1987
Man	Brain: ipselateral jugular venous plasma	Ischaemia: unilateral internal carotid ligation	0.06 to 0.22	Sollevi, 1986
Dog	Hindlimb: venous plasma	Exercise: stimulated at 6 Hz	0.06 to 0.14	Fuchs et al., 1986
Dog	Hindlimb: venous plasma	Ischaemic contraction: 5 min	0.04 to 0.22	Dobson et al., 1971
Rat	Liver: perfusate	Hypoxia	0.05 to 1.1	Arnold & Cysyk, 1986

*The left hand figure represents the basal value and the right hand figure the value in response to the stimulus indicated.

CHAPTER 7 - ACUTE HAEMODYNAMIC CHANGES DURING INTRAVENOUS INFUSION OF ADENOSINE

7.1 - INTRODUCTION

The studies described so far in this thesis have shown that infusion of adenosine in conscious subjects produces haemodynamic as well as respiratory changes. Although often considered separately the cardiovascular and respiratory systems are intimately related both anatomically and physiologically. Changes in one system can affect the other. It would therefore be expected that, in view of its marked effects on ventilation, however caused, adenosine infusion would produce haemodynamic changes. Furthermore adenosine has been shown to cause local changes in the cardiovascular system including potent vasodilation in many tissues as discussed in Chapter 1. Such changes and their systemic haemodynamic consequences might affect respiration.

Whether and in what circumstances adenosine could be useful as a respiratory stimulant depends in part on the nature and magnitude of its cardiovascular effects.

Adenosine has been used to induce hypotension in patients undergoing intracranial surgery (Sollevi et al., 1984b; Öwall et al., 1987) and detailed studies of its haemodynamic effects in anaesthetised patients have been performed. Sollevi (1986) suggested that adenosine might be useful for reducing afterload in patients with heart failure. However, as shown in this thesis, infusion of adenosine into conscious subjects produces effects not seen during anaesthesia, eg) marked stimulation of respiration and various symptoms. In addition the patterns of change in heart rate and blood pressure in conscious subjects differ from those reported in anaesthetised patients.

Since the haemodynamic effects of adenosine infusion and their relationship to the respiratory changes in conscious subjects had not previously been fully defined they were the subject of this study.

7.2 - METHODS

7.2.1 - PATIENTS AND INFUSIONS

The subjects were 16 patients (11 men), aged 37 to 64 (mean: 52) years and weighing 63 to 95 (mean: 78) kg, undergoing cardiac catheterisation for investigation of chest pain (Table 7.1). Patients with unstable angina or heart failure were excluded. Some patients suspected of having non-cardiac chest pain who nevertheless required coronary arteriography were included: 9 patients had normal coronary arteriograms. Exercise stress tests which had been performed in 8 of these patients were negative. Although microvascular angina was not specifically excluded, all 9 patients with normal arteriograms were ultimately considered to have non-cardiac pain. The other 7 patients had coronary artery disease (Table 7.1).

Patients were receiving a variety of medications as shown in Table 7.1. In 3 patients treatment with a ß-adrenoceptor antagonist (atenolol) had been discontinued 30 - 45 h previously. All patients received diazepam 10mg orally one hour before catheterisation. All had abstained from caffeine-containing beverages for at least 6 h.

After baseline measurements solutions were infused single blind via the femoral venous sheath. Placebo (0.9% sodium chloride) was given for 5 min, followed by adenosine with dosage increments every 5 min except in the case of the first adenosine stage which was continued for 6 min to ensure clearing of the dead-space of the venous sheath. An initial dose of adenosine of 4.3 mg/min was used because studies previously described in this thesis suggested that such a dose would produce mild effects. Subsequent doses were 6.1, 8.5, 11.9 mg/min as tolerated. At the end of each stage, patients were asked to report any symptoms and if they were willing to continue to the next dose.

Table 7.1 - Patient characteristics.

Patient	Age (years)	Sex	Height (m)	Weight (kg)	Treatment	Diagnosis
1	56	M	1.73	85	Z	3-VD
2	63	M	1.69	72	B C N	3-VD
3	45	M	1.75	80	B C	1-VD
4	60	M	1.71	90	B CDN	2-VD
5	50	M	1.73	72	Z	Musculoskeletal pain
6	39	F	1.64	62	-	Oesophageal spasm
7	37	F	1.63	71	B	Musculoskeletal pain
8	52	F	1.61	80	-	Musculoskeletal pain
9	51	M	1.77	94	-	Musculoskeletal pain
10	64	M	1.69	71	B*C NE	Oesophageal spasm
11	62	F	1.60	70	B* L	Hyperventilation
12	54	M	1.87	95	C	2-VD
13	55	M	1.63	78	-	Hyperventilation
14	44	F	1.52	60	B*	1-VD
15	55	M	1.84	83	A	Musculoskeletal pain
16	47	M	1.70	85	B C	1-VD
Mean	52	-	1.69	78	-	-
SD	8	-	0.09	10	-	-

M, male; F, female; 1-VD, 1 vessel coronary artery disease; 2-VD, 2 vessel disease; 3-VD, 3 vessel disease; B, ß-adrenoceptor antagonist; *withdrawn at least 30 h before the study; C, calcium antagonist; D, diuretic; N, long-acting nitrate; A, amiodarone; E, etrinate; L, lederfen; Z, benzodiazepine.

7.2.2 - MEASUREMENTS AND SAMPLES

The study was performed following an equilibration period of at least 10 min after insertion of catheters and before any angiograms. Except as indicated below measured variables were recorded following the equilibration period (baseline), during the final 3.5 min of each

infusion stage and 5 min following the end of the final infusion stage. The first adenosine stage was continued for 2.5 min prior to measurements being made to allow clearing of the deadspace of the infusion sheath. It was reasoned that steady-state concentrations of adenosine would be achieved quickly because of its very short half-life and because at the doses used recirculating venous concentrations are unchanged as shown in Chapter 5.

An ECG was recorded throughout using a single bipolar lead (modified standard lead 2) and was used to determine heart rate and PR interval.

A pigtail catheter (7F gauge) was inserted through an 8F sheath in the right femoral artery for measurement of systemic and, in some patients, left ventricular pressures. A balloon tipped catheter (Swan-Ganz, 7F gauge) was inserted via a 9F sheath in the right femoral vein and positioned for measurement of right atrial pressure, pulmonary artery pressure and pulmonary capillary wedge pressure (Swan et al., 1970). Pressures (mean of at least five non-ectopic beats) were recorded via the catheters which were connected to Bell and Howell strain gauge transducers, a Cambridge amplifier, and a Mingograph multichannel recorder (8 patients), or to Hewlett Packard quartz transducers and amplifier and a Gould recorder (8 patients). Patients were supine and the zero reference level was at the sternal angle. Arterial blood pressure was not measured in 2 patients (nos. 6 and 7) because of a transducer fault. In the last 8 patients (3 with coronary disease) left ventricular end-diastolic pressure was also measured at baseline and during the final infusion stage.

Cardiac output (mean of at least 3 estimations) was measured by the thermodilution technique (Ganz et al., 1971). Ten ml of ice-cold 5% dextrose solution was rapidly injected via the lumen of the Swan-Ganz catheter which terminated in the right atrium. The temperature transient was detected by a thermistor at the tip of the

catheter which was situated in the pulmonary artery. Cardiac output was calculated on-line using an Instrumentation Laboratories computer.

Respiration was recorded using a respiratory inductance plethysmograph in the first 12 patients. Isovolume manoeuvres performed by each patient were used to determine the relative contributions of the abdominal and ribcage signals. Volume calibration was then performed using a spirometer (Micro Medical Instruments). A spirometer fault prevented this in the first case. Respiratory rate, tidal volume and minute ventilation were determined from the respiratory trace, recorded on a chart recorder.

Blood (1ml) was sampled from the pulmonary artery and aorta at each stage for determination of oxygen saturation (OSM2 Hemoximeter, Radiometer, Copenhagen). Samples (2ml) were also obtained in the last 8 patients at baseline, during the final infusion stage, and 5 min post-infusion, for determination of arterial blood gas tensions and pH (Ciba-Corning Model 178 Blood Gas Analyser).

Derived haemodynamic indices were calculated using standard formulae (WHO/ISFC Task Force on Haemodynamics, 1985), as follows:

$$PVR = \frac{\text{Mean PAP - Mean PCWP}}{CO} \times 80 \qquad \text{(Eq. 7.1)}$$

$$SVR = \frac{\text{Mean BP - Mean RAP}}{CO} \times 80 \qquad \text{(Eq. 7.2)}$$

$$SVI = \frac{CI \times 1000}{HR} \qquad \text{(Eq. 7.3)}$$

Where PVR and SVR are pulmonary and systemic vascular resistances respectively (dyne.s.cm^{-5}), PAP is pulmonary artery pressure, PCWP is pulmonary capillary wedge pressure, BP is systemic blood pressure and RAP is right atrial pressure (mmHg), CO is cardiac output (l/min), CI is cardiac index (l/min/m^2), SVI is stroke volume index

(ml/beat/m^2) and HR is heart rate (beats/min).

Total body oxygen consumption (VO_2; ml/min) was calculated from arteriovenous oxygen content difference ($AV\text{-}O_2cont$; ml/l) and cardiac output (CO; l/min) as follows (Rubin et al., 1982):

$$VO_2 = CO \times AV\text{-}O_2cont \qquad \text{(Eq. 7.4)}$$

$AV\text{-}O_2cont$ was calculated from the arteriovenous difference in oxygen saturation ($AV\text{-}O_2sat$; %) and haemoglobin concentration (Hb; g/dl) as follows (WHO/ISFC Task Force on Haemodynamics, 1985):

$$AV\text{-}O_2cont = 1.34 \times AV\text{-}O_2sat \times Hb \qquad \text{(Eq. 7.5)}$$

where 1.34 is the empirical value of oxygen capacity of a gram of haemoglobin.

7.2.3 - STATISTICAL ANALYSIS

Repeated measures analysis of variance and the Student-Newman-Keuls test, or the paired t-test, where appropriate, were used to analyse changes in measured variables. Differences between subgroups according to diagnosis and treatment were compared using the unpaired t-test, the Mann-Whitney u-test or the Kruskal-Wallis test for non-parametric analysis of variance, as appropriate. Correlations were examined using the product-moment or Spearman rank correlation coefficients.

7.3 - RESULTS

The maximum dose of adenosine given ranged from 6.1 to 11.9 (mean: 8.5, (SD: 2.3) mg/min, equivalent to 107.9 (SD: 24.3) μg/kg/min) and correlated with both body weight ($r = 0.60$, $P < 0.02$) and body surface area ($r = 0.74$, $P < 0.001$). All patients received at least two doses, 4.3 and 6.1 mg/min, equivalent to 56.5 (SD: 7.8) and 79.3 (SD: 10.9) μg/kg/min.

7.3.1 - EFFECTS OF PLACEBO

There were no significant changes during the placebo

saline infusion (Figs. 7.1 to 7.5 and Table 7.2).

7.3.2 - HAEMODYNAMIC AND RESPIRATORY EFFECTS OF ADENOSINE

a) Lowest Dose

At the lowest dose of adenosine pulmonary vascular resistance fell by 20% (Fig. 7.3), but there were no other significant haemodynamic changes (Fig. 7.1 to 7.3). Minute ventilation, however, increased by 44% (Fig. 7.5).

b) Maximum Dose

Changes during the maximum dose are summarised in Table 7.2. At the maximum dose adenosine infusion increased heart rate by 34% but did not affect systolic, diastolic or mean systemic blood pressure (Fig. 7.1). PR interval was unchanged. Cardiac index increased by 52% and stroke index by 12% (Fig. 7.1). Mean pulmonary capillary wedge pressure and pulmonary artery pressure both increased by 6 (SD: 5) mmHg to 10 (SD: 5) and 16 (SD: 5) mmHg respectively. Mean right atrial pressure did not change significantly (Fig. 7.2). Pulmonary and systemic vascular resistances fell, by 38% and 34% respectively (Fig. 7.3). Total oxygen consumption showed no significant change (Fig. 7.4). The product of systolic blood pressure and heart rate, an approximate index of myocardial work (Gobel et al., 1978), increased by 28% (Fig. 7.4).

Left ventricular end-diastolic pressure, measured in 8 patients, increased by 9 (SD: 8) mmHg to 14 (SD: 10) mmHg. The change in pulmonary capillary wedge pressure in this group, 3 (SD: 2) to 9 (SD: 6) mmHg ($P < 0.05$), was similar to the change in the 8 patients (4 with coronary artery disease) in whom left ventricular end-diastolic pressure was not measured directly: 3 (SD: 3) to 10 (SD: 5) mmHg, $P < 0.001$.

Minute ventilation increased by 84%, due largely to a 60% increase in tidal volume (Fig. 7.5). The arterial carbon dioxide tension fell by 8 (SD: 4) mmHg to 31 (SD:

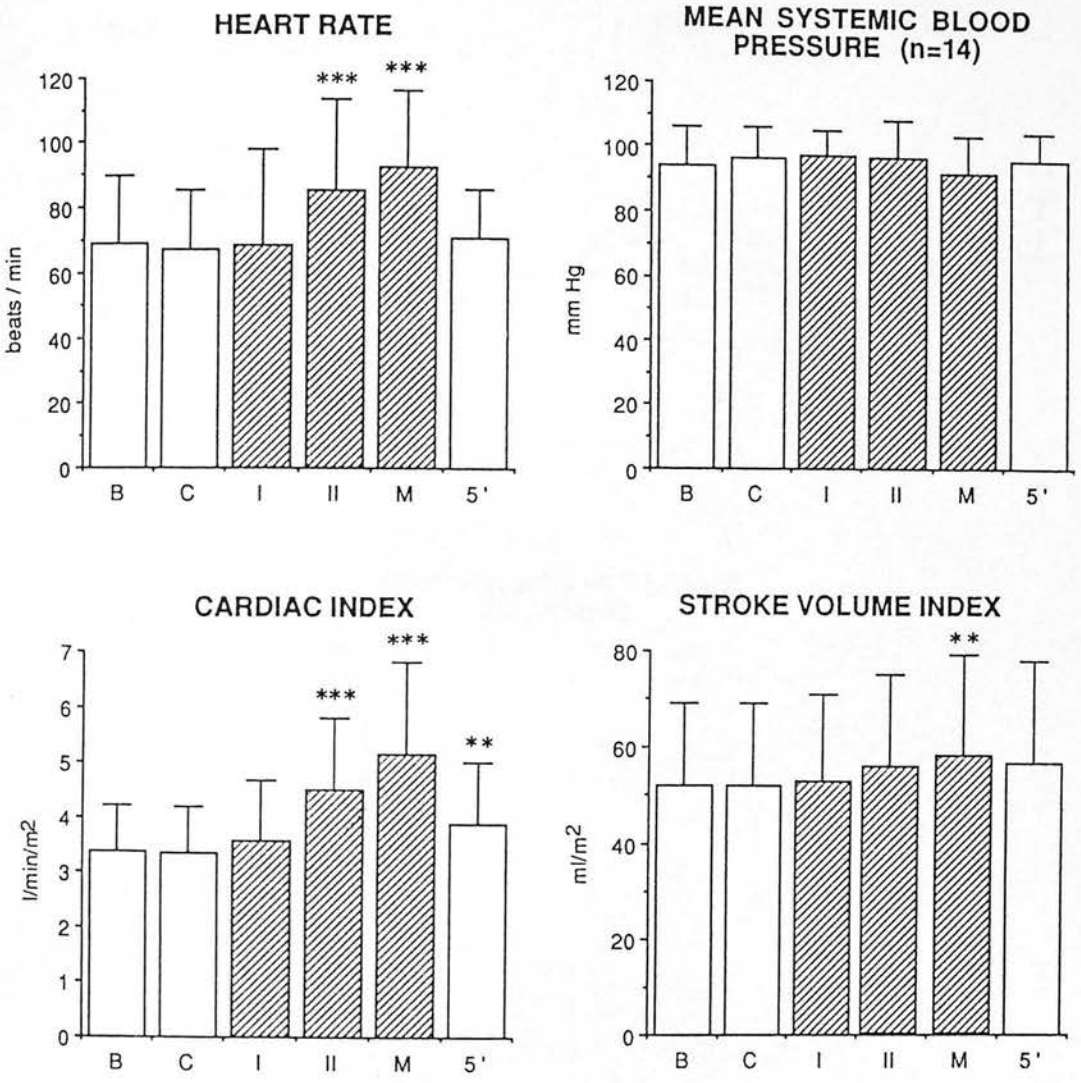

Fig. 7.1 - Changes in heart rate, blood pressure, cardiac index and stroke volume index during adenosine infusion.

B, baseline; C, control infusion; I, adenosine infusion at 4.3 mg/min; II, adenosine infusion at 6.1 mg/min; M, maximum dose of adenosine for each subject; 5', 5 min following adenosine infusion; $^{**}P < 0.01$, $^{***}P < 0.001$ for comparisons with baseline. n = 16 except where shown.

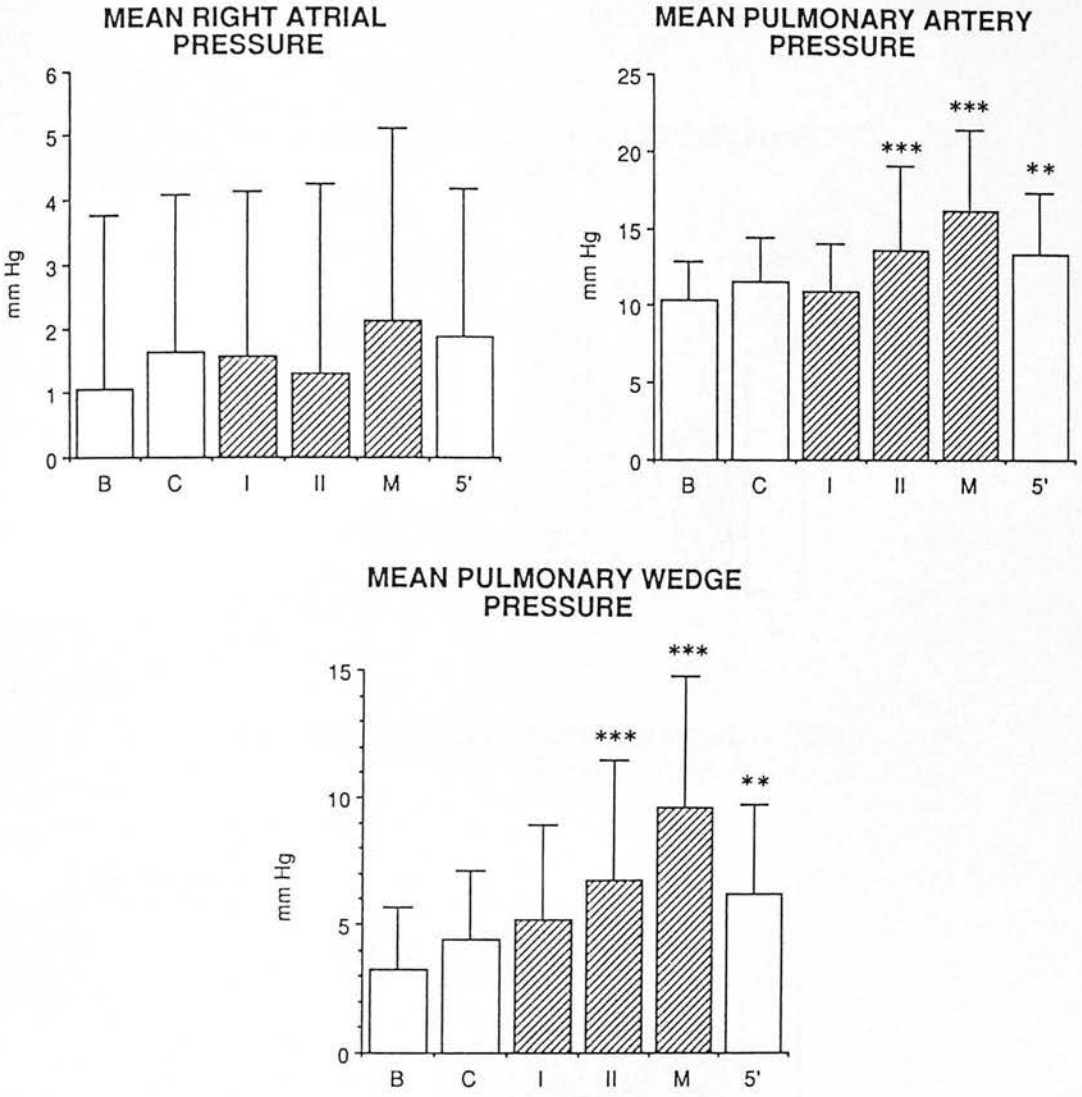

Fig. 7.2 - Changes in mean right atrial, pulmonary artery and pulmonary capillary wedge pressures during adenosine infusion. Symbols and abbreviations as in Fig. 7.1. n = 16.

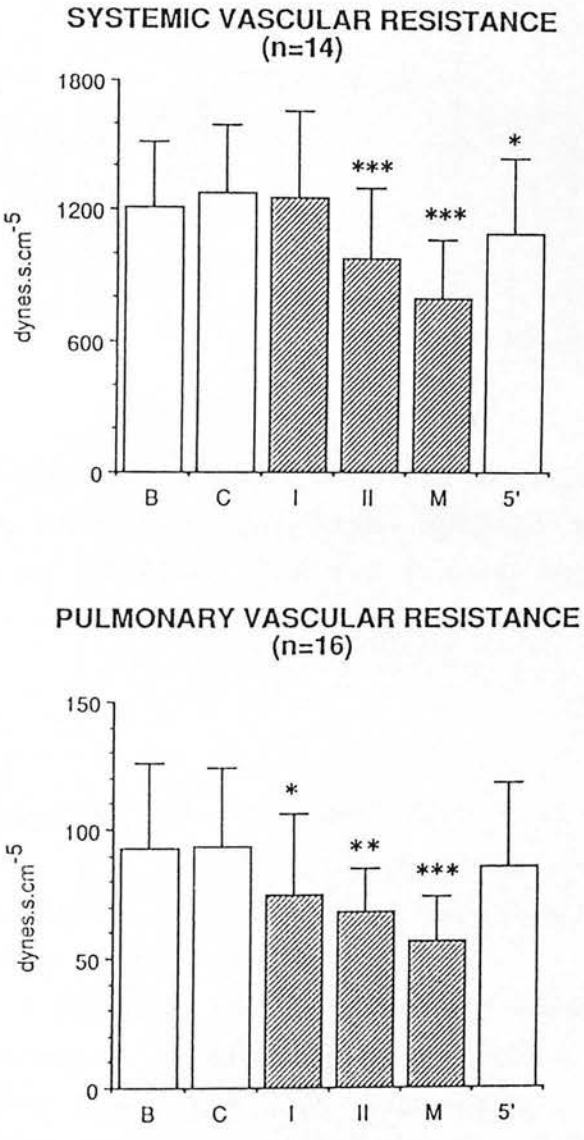

Fig. 7.3 - Changes in systemic and pulmonary vascular resistance during adenosine infusion. *P < 0.05. Other symbols and abbreviations as in Fig. 7.1.

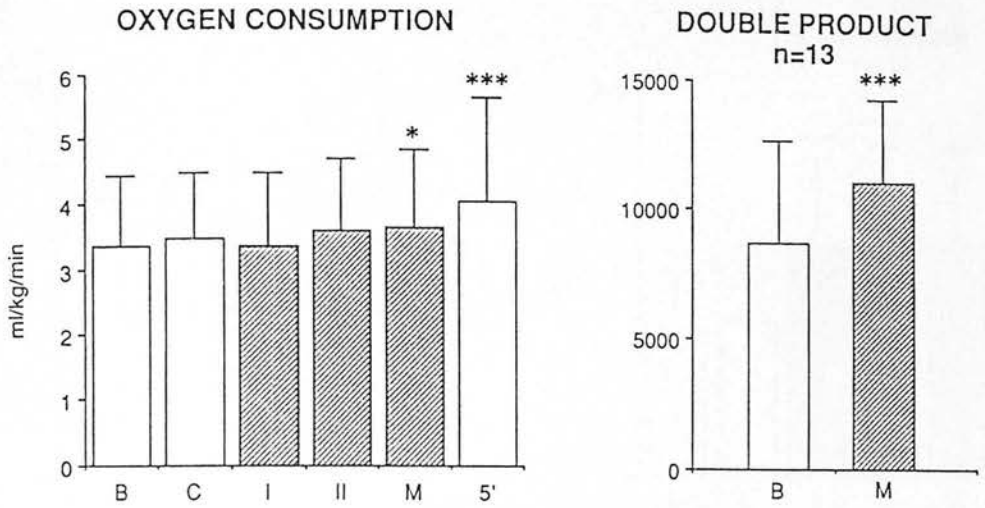

Fig. 7.4 - Changes in oxygen consumption and double product during adenosine infusion. Symbols and abbreviations as in Figs. 7.1 and 7.3. n = 16 except where shown.

3) mmHg and arterial pH increased from 7.39 (SD: 0.03) to 7.45 (SD: 0.02). The arterial oxygen tension increased slightly (by 2 mmHg), but this was not statistically significant (Fig. 7.5).

There was a significant correlation between the percentage increases in heart rate and minute ventilation (r_S = 0.615, P < 0.05, n = 12), but neither of these correlated significantly with the percentage decrease in either pulmonary vascular resistance (r_S = 0.165, n = 16 and r_S = -0.406, n = 12, respectively) or systemic vascular resistance (r_S = 0.297, n = 14 and r_S = -0.091, n = 10, respectively).

c) Highest Dose Without Symptoms

Ten patients received at least 1 dose of adenosine without experiencing symptoms. In these patients the

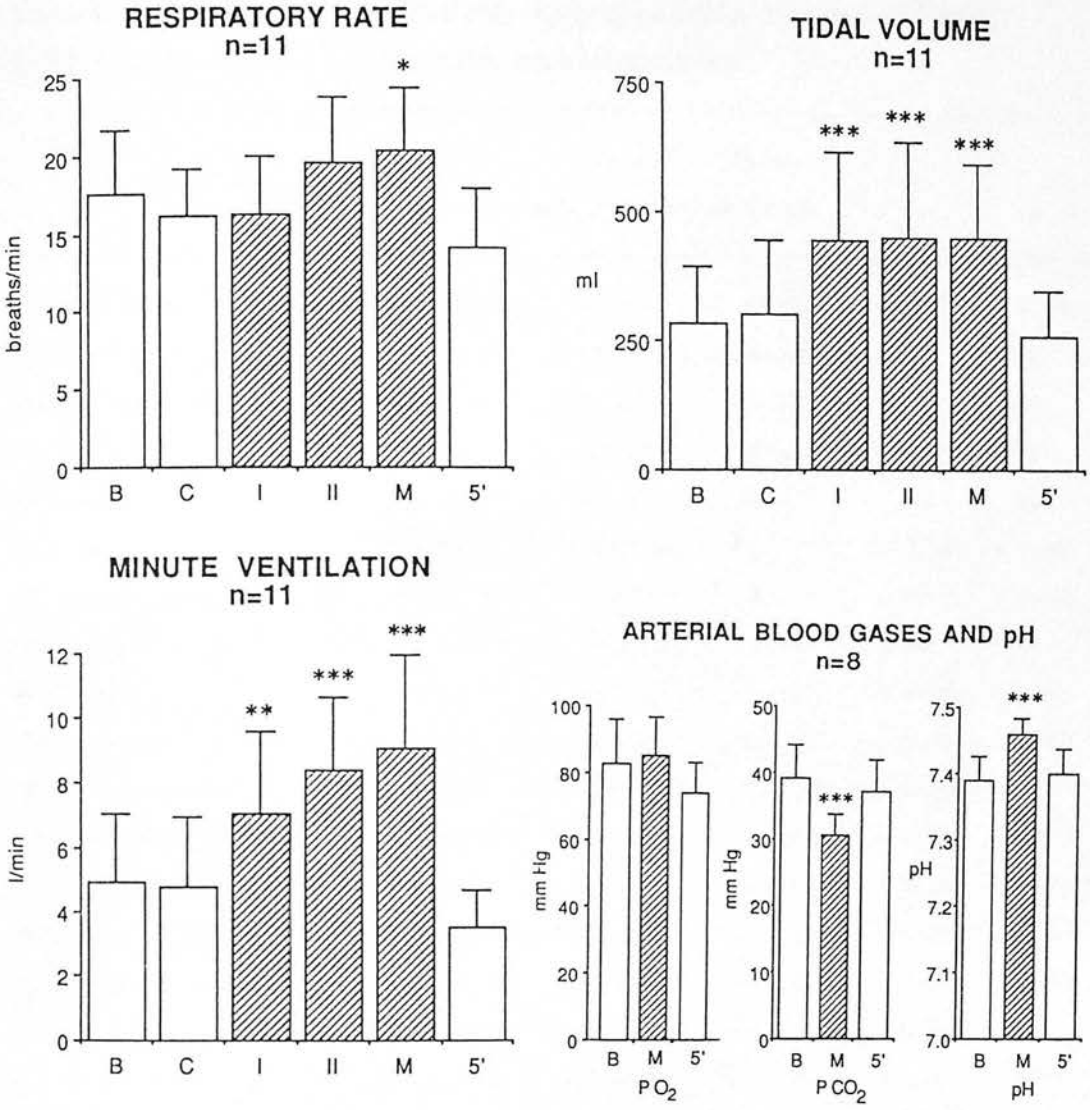

Fig. 7.5 - Changes in respiration, blood gas tensions and pH during adenosine infusion. Symbols and abbreviations as in Figs. 7.1 and 7.3.

Table 7.2 - Changes during the placebo infusion and adenosine infusion at the maximum dose.

	Baseline	Placebo	Adenosine	P*	
	(n = 16 except where indicated)			a	b
HR (beats/min)	69 (21)	67 (19)	92 (24)	< 0.001	< 0.001
PR interval (s)(n=14)	0.18 (0.02)	0.18 (0.02)	0.19 (0.02)	NS	NS
SBP (mmHg)(n=13)	127 (20)	134 (16)	124 (16)	NS	NS
DBP (mmHg)(n=13)	70 (8)	73 (6)	67 (8)	NS	NS
MBP (mmHg)(n=14)	94 (12)	96 (9)	92 (11)	NS	NS
CI ($l/min/m^2$)	3.4 (0.8)	3.3 (0.8)	5.1 (1.7)	< 0.001	< 0.001
SVI ($ml/beat/m^2$)	52 (17)	52 (17)	58 (21)	< 0.01	< 0.001
RAP (mmHg)	1 (3)	2 (2)	2 (3)	NS	NS
PAP (mmHg)	10 (3)	12 (3)	16 (5)	< 0.001	< 0.001
PCWP (mmHg)	3 (2)	4 (3)	10 (5)	< 0.001	< 0.001
LVEDP (mmHg)(n=8)	5 (6)	-	14 (10)	< 0.05	-
SVR ($dyne.s.cm^{-5}$)(n=14)	1209 (299)	1270 (319)	794 (267)	< 0.001	< 0.001
PVR ($dyne.s.cm^{-5}$)	93 (32)	94 (30)	58 (18)	< 0.001	< 0.001
RPP (mmHg.beats/min)	8647 (4018)	8640 (3261)	11048 (3248)	< 0.001	< 0.001
f_R (breaths/min)(n=11)	18 (4)	16 (3)	20 (4)	< 0.05	< 0.01
V_T (l)(n=11)	0.28 (0.11)	0.30 (0.14)	0.45 (0.15)	< 0.001	< 0.001
V (l/min)(n=11)	4.9 (2.1)	4.8 (2.1)	9.9 (2.9)	< 0.001	< 0.001
VO_2 ($ml/kg/m^2$)	3.4 (1.1)#	3.5 (1.0)	3.7 (1.2)	NS	NS
PaO_2 (mmHg)(n=8)	83 (13)	-	85 (12)	NS	-
$PaCO_2$ (mmHg)(n=8)	39 (5)	-	31 (3)	< 0.001	-
Arterial pH (n=8)	7.39 (0.03)	-	7.46 (0.02)	< 0.001	-

Data are mean (SD). *P values refer to: (a) adenosine vs. baseline and (b) adenosine vs. placebo. NS, not significant; HR, heart rate; SBP, systolic arterial blood pressure; DBP, diastolic blood pressure; MBP, mean arterial blood pressure; CI, cardiac index; SVI, stroke volume; RAP, mean right atrial; PAP, mean pulmonary artery pressure; PCWP, mean pulmonary capillary wedge pressure; SVR, systemic vascular resistance; PVR, pulmonary arteriolar resistance; RPP, rate-pressure product; f_R, respiratory frequency; V_T, tidal volume; V, minute ventilation; VO_2, total oxygen consumption; PaO_2, arterial oxygen tension; $PaCO_2$, arterial carbon dioxide tension; #n = 15.

Table 7.3 - Changes during adenosine infusion at the maximum dose given without causing symptoms.

	Baseline	Placebo	Adenosine	P*	
	(n = 10 except where indicated)			a	b
HR (beats/min)	61 (8)	59 (7)	62 (8)	NS	NS
CI (l/min/m²)	3.4 (0.9)	3.3 (0.8)	3.6 (1.4)	NS	NS
PAP (mmHg)	10 (2)	12 (3)	10 (3)	NS	NS
PCWP (mmHg)	3 (3)	4 (3)	4 (3)	NS	NS
PVR (dyne.s.cm^{-5})	96 (30)	99 (28)	76 (28)	< 0.05	< 0.05
SVR (dyne.s.cm^{-5}) (n=9)	1,104 (280)	1,173 (306)	1,168 (400)	NS	NS
V (l/min)(n=8)	5.4 (2.4)	5.2 (2.3)	6.7 (2.2)	NS	NS

Data are mean (SD). *P values refer to: (a) adenosine vs. baseline and (b) adenosine vs. placebo. NS = not significant. Abbreviations as in Table 7.2.

maximum dose without symptoms, 4.7 (SD: 0.7, range: 4.3 to 6.1) mg/min, caused a 21% fall in pulmonary vascular resistance but no other significant changes (Table 7.3).

7.3.3 - SYMPTOMS CAUSED BY ADENOSINE

The dose of adenosine at which symptoms were first reported varied (Fig. 7.6). Precordial discomfort was reported by 14 patients: 6 of the 7 with coronary disease and 8 of the 9 with normal angiography. Ten patients compared the character of the discomfort with their usual chest pain: 6 (3 with coronary disease, 3 without) thought it was the same and 4 (2 with coronary disease, 2 without) thought it was different. Two patients who experienced their usual chest pain developed 1 mm ST segment depression. One had normal angiography, the other two-vessel disease.

Dyspnoea was reported by 11 patients and usually first occurred at higher doses than precordial discomfort (Fig. 7.6). Other symptoms included flushing of the head

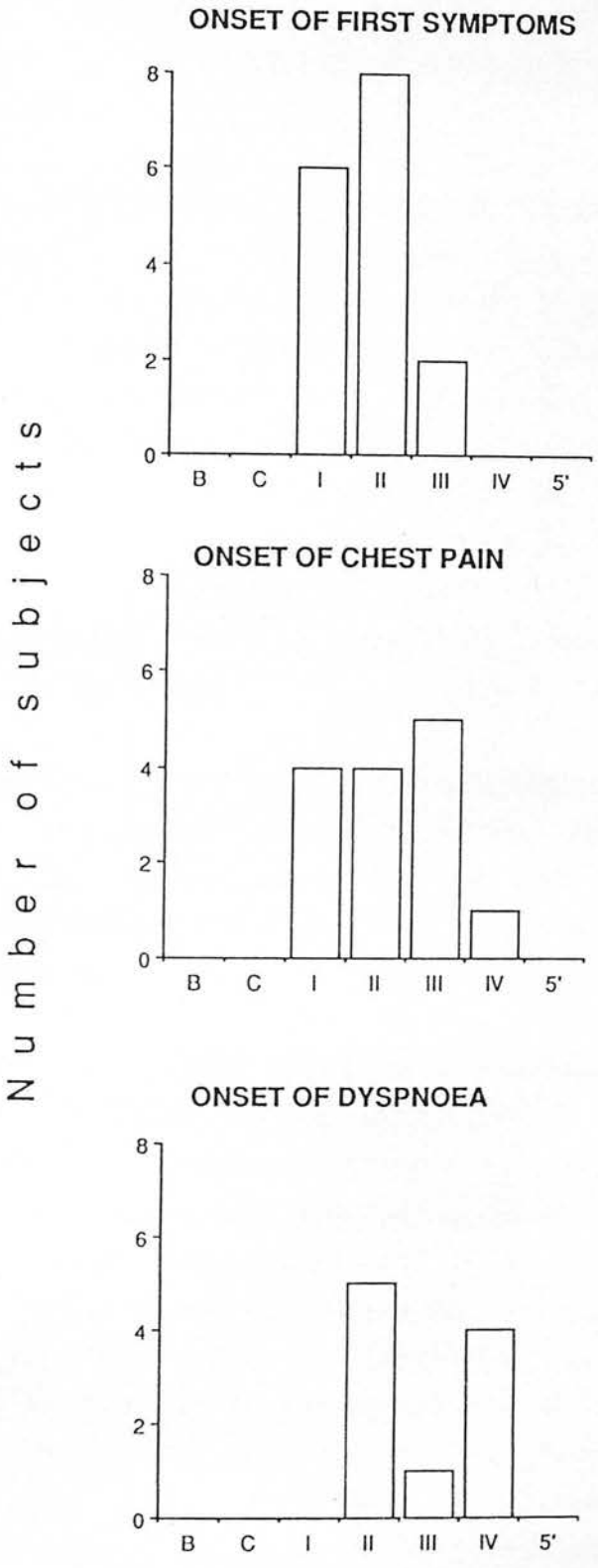

Fig. 7.6 - Onset of symptoms during the study. III, adenosine infusion at 8.5 mg/min; IV, adenosine infusion at 11.9 mg/min. Other symbols as in Fig. 7.1.

or neck in 6 patients (with another 6 visibly flushed), headache in 4, lightheadedness in 3 and epigastric discomfort in 1.

7.3.4 - CHANGES FOLLOWING ADENOSINE INFUSION

Most variables were returning towards baseline values 5 min after the infusion (Figs. 7.1 to 7.3). Minute ventilation tended to fall below the baseline value (by 28%) and this was associated with a fall in arterial oxygen tension to 74 (SD: 9.5) mmHg, but these changes were not statistically significant (Fig. 7.5). Oxygen consumption increased to 121% of the baseline value 5 min after the infusion ($P < 0.01$) (Fig. 7.4).

All symptoms resolved completely within 1 to 2 min of stopping the infusion.

7.3.5 - EFFECTS OF DIAGNOSIS ON RESPONSES TO ADENOSINE

There were no significant differences, in baseline values, or changes during adenosine infusion, of any variable, between patients with and without coronary artery disease.

7.3.6 - EFFECTS OF TREATMENT WITH ß-ADRENOCEPTOR ANTAGONISTS ON RESPONSES TO ADENOSINE

In the 3 patients whose ß-adrenoceptor antagonist treatment, atenolol, was discontinued between 30 and 45 h before the study, baseline heart rate, 93 (SD: 28) beats/min, was significantly higher than the value, 55 (SD: 8) beats/min, in those continuing treatment ($P < 0.05$), and tended to be higher than the value, 69 (SD: 17) beats/min, in those who had never received such treatment (NS). There were no other differences in baseline variables between these three groups.

The rise in left ventricular end-diastolic pressure, during adenosine infusion, in patients in whom atenolol was recently withdrawn, 2 (SD: 4) mmHg ($n = 3$), was significantly smaller than the change, 14 (SD: 6) mmHg ($n = 4$),in those who had not received such a drug $P < 0.05$)

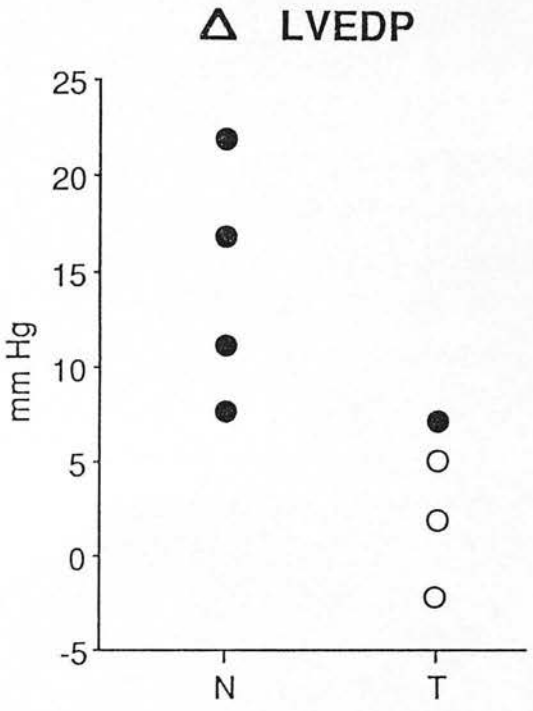

Fig. 7.7 - Change in left ventricular end-diastolic pressure in 8 subjects during adenosine infusion at the maximum dose. N, not treated with ß-adrenoceptor blockers; T, treated with ß-adrenoceptor blockers: open symbols indicate patients in whom the treatment was discontinued at least 30 hours prior to the study.

(Fig. 7.7). The adenosine-induced increase in pulmonary capillary wedge pressure in those patients in whom atenolol had recently been withdrawn, 2.0 (SD: 4.4) mmHg (n = 3), was likewise smaller, though not significantly so, than the changes, 7.9 (SD: 4.9) mmHg (n = 8) and 6.6 (SD: 2.6) mmHg (n = 5), in those patients who had never received such a drug or who continued treatment respectively. There were no other significant differences during adenosine infusion between the three groups. In particular there were no differences in the heart rate increment between patients not receiving a ß-adrenoceptor antagonist, those who were ß-blocked and those in whom such treatment had recently been withdrawn (24 (SD: 9, n

= 8), 21 (SD: 7, n = 5) and 26 (SD: 8, n = 3) beats/min respectively).

7.4 - DISCUSSION

This study demonstrates that in conscious subjects intravenous infusion of adenosine causes both systemic and pulmonary vasodilation. At the lowest dose used, there was a selective effect on the pulmonary circulation. At higher doses, adenosine also increased left ventricular filling pressure, an effect not previously described.

Results of some previous detailed studies of the haemodynamic effects of adenosine in anaesthetised animals and man are summarised in Tables 7.4 and 7.5. Universal findings have been that adenosine causes systemic vasodilation, a reduction in systemic blood pressure, little change in right atrial, pulmonary artery or pulmonary capillary wedge pressures and increased coronary flow. Heart rate and cardiac output responded variably in animals, but both were increased in man, although the changes in heart rate were small. Pulmonary vascular resistance was consistently reduced in man (see below), albeit not always to a statistically significant degree. Stroke work, where measured, has been shown to fall. Myocardial oxygen consumption increased in two animal studies in both of which adenosine caused substantial increases in heart rate. Responses in man have been variable. Changes in total oxygen consumption have been small. Where measured PaO_2 has been shown to decrease.

Compared with the findings in anaesthetised patients the main differences observed in this study in conscious subjects were: greater increases in heart rate and cardiac output, no change in systemic blood pressure and a rise in left ventricular filling pressure.

Table 7.4 - Haemodynamic changes during adenosine infusion in anaesthetised animals.

	Rowe et al. 1962	Afonso et al. 1970	Kassell et al. 1983[1]	Lagerkranser et al. 1984	Boarini et al. 1985	Norlén et al. 1988	Öwall et al. 1986
Animals	dogs	8 dogs	6 dogs	10 dogs	12 dogs	9 pigs	9 dogs
Adenosine dose[2]	534[3]	mean: 10.4 mg/min[3]	mean: 50[4]	range: 187-4245[5]	-[6]	mean: 3071[7]	mean: 1360[8]
HR (beats/min)	+60***	+47***	-40***	-34**	-33***	-10	-33**
BP (mmHg)	-10*	-13**	-64***	-57**	-60***	-44***	-32***
CO or CI (%)	+46***	+59***	-8	+22*	-25	+11	+1
SV or SVI (%)	-	-	+48	+77**	0	+29**	+31
CBF (%)	+574*	+307**	-	-	+45	+99	+35*
RAP (mmHg)	-0.9	-	-	+2**	-2	+0.4	-
PAP (mmHg)	0	+1	-	-	-2	-0.7	-
PCWP or LVEDP (mmHg)	-	-	-	+1.2*	0	-0.5	-3**
SVR or SVRI (%)	-39**	-43***	-67**	-62**	-50***	-58***	-
TPR (%)	-29***	-34**	-	-	-	-	-
CVR (%)	-71	-	-	-	-	-	-53*
LVSW (%)	-	-	-56*	-	-63**	-	-
MVO_2 (%)	+113	+53***	-	-	-	-	-25***
VO_2 (%)	+17	+16*	-	-14**	-	-	-
PaO_2 (mmHg)	-	-	-	-5	-	-3	-

Haemodynamic data are shown as the change in the mean value compared with control. HR, heart rate; BP, mean systemic arterial blood pressure; CO, cardiac output; CI, cardiac index; SV(I), stroke volume (index); CBF, regional coronary blood flow; RAP, mean right atrial or central venous pressure; PAP, mean pulmonary artery pressure; PCWP, mean pulmonary capillary wedge pressure; LVEDP, left ventricular end-diastolic pressure; SVR(I), systemic vascular resistance (index); TPR, total pulmonary resistance; CVR, coronary vascular resistance; LVSW, left ventricular stroke work; MVO_2, myocardial oxygen consumption; VO_2, total oxygen consumption; PaO_2, arterial oxygen tension. [1]90 min values; [2]μg/kg/min except where shown; [3]into right atrium; [4]site not specified, dipyridamole pretreatment; [5]ascending aorta or inferior vena cava, dipyridamole pretreatment (n = 4); [6]dose and site not specified; [7]site not specified; [8]femoral vein; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Table 7.5 - Haemodynamic changes during adenosine infusion in anaesthetised patients.

	Sollevi *et* 1984b	Öwall *et al.* 1987	Öwall *et al.* 1988b	Öwall *et al.* 1988a	Zäll *et al.* 1989
Patients	10	47	5	6	14
Procedure	intracranial vascular surgery. controlled hypocapnia	intracranial vascular surgery. controlled hypocapnia	intracranial vascular surgery. controlled hypocapnia	abdominal aortic aneurysm surgery	coronary artery grafting
Other drugs	diazepam atropine droperidol thiopentone phenoperidine pancuronium mannitol N_2O Dipyridamole	diazepam atropine droperidol thiopentone phenoperidine pancuronium mannitol N_2O	diazepam atropine droperidol thiopentone phenoperidine pancuronium mannitol N_2O	morphine scopolamine fentanyl pancuronium	fentanyl droperidol N_2O pancuronium ß-blockers
Adenosine dose (µg/kg/min)[1]	140 (40)[2]	214 (18)	217 (32)	90 (20)	120
HR (beats/min)	+9**	+8**	+4	+2	+10**
BP (mmHg)	-36**	-34**	-23**	-19**	-14**
CO or CI (%)	+39**	+38**[3]	+41*	+19*	+56**
SVI (%)	-	-	+28	+12	+25**
CSBF (%)	-	-	+75**	+121**	+122**
RAP (mmHg)	+0.4	-0.8**	+1	+1	+0.4
PAP (mmHg)	+2.1	+1[3]	+2	-1	-
PCWP (mmHg)	+1.4	0[3]	+2	-1	-0.3
SVR or SVRI (%)	-63**	-65**[3]	-50**	-37**	-43**
PVR or PVRI (%)	-15	-32*[3]	-28	-33*	-31**
CVR (%)	-	-	-67**	-65**	-
LVSWI (%)	-	-	-22	-12	-
RVSWI (%)	-	-	+40*	-7	-
MVO_2 (%)	-	-	-38*	+9	-13
VO_2 (%)	-12*	-	-11	-6	-
PaO_2 (mmHg)	-14	-18*[4]	-4	-21*	-

Haemodynamic variables are expressed as the change in the mean value compared with control. HR, heart rate; BP, mean systemic arterial blood pressure; CO, cardiac output; CI, cardiac index; SVI, stroke volume index; CSBF, coronary sinus blood flow; RAP, mean right atrial or central venous pressure; PAP, mean pulmonary artery pressure; PCWP, mean pulmonary capillary wedge pressure; SVR(I), systemic vascular resistance (index); PVR(I), pulmonary vascular resistance (index); CVR, coronary vascular resistance; LVSWI, left ventricular stroke work index; RVSWI, right ventricular stroke work index; MVO_2, myocardial oxygen consumption; VO_2, total oxygen consumption; PaO_2, arterial oxygen tension. [1]infused into superior vena cava or right atrium, mean (SEM); [2]patients pretreated with dipyridamole; [3]n = 7; [4]n = 21; *P < 0.05; **P < 0.01.

7.4.1 - EFFECTS OF ADENOSINE ON SYSTEMIC AND PULMONARY VASCULAR RESISTANCE

Although adenosine has been used as a systemic vasodilator during anaesthesia (Sollevi et al., 1984; Öwall et al., 1987), the present study showed that in conscious subjects infusion rates of adenosine sufficient to reduce peripheral vascular resistance usually cause symptoms. This may limit the clinical use of adenosine as a systemic vasodilator. Bush et al. (1989) recently reported that adenosine infusion at 70 μg/kg/min increased effective pulmonary blood flow and reduced estimated systemic vascular resistance whereas infusion at 30 μg/kg/min was without effect. Most of their subjects experienced side-effects at 70 μg/kg/min and six said they would not have tolerated a higher dose. Edlund et al. (1990) showed in healthy volunteers that adenosine infusion into a central vein at rates up to 80 μg/kg/min caused reduction of more than 50% in systemic vascular resistance associated with a doubling of cardiac output. They also observed a 28%, but not significant, reduction in pulmonary vascular resistance. All of their subjects developed side-effects and only 5 were willing to receive the maximum infusion rate.

The selective effect of adenosine on the pulmonary circulation at lower doses observed in the present study might be due to lower concentrations reaching the systemic circulation because of rapid uptake of adenosine by endothelial (Catravas, 1984) and red blood cells (Klabunde, 1983).

Previous reports of the effects of adenosine on the pulmonary vasculature have been conflicting. Drury and Szent-Györgyi (1929) observed that adenosine increased pulmonary artery pressure in a dog heart-lung preparation. Bennet and Drury (1931) inferred that adenosine caused pulmonary vasoconstriction from rather crude experiments in perfused rabbit lungs. Gaddum & Holz (1933) showed that in isolated perfused cat lungs small doses of adenosine produced transient vasodilation while larger

doses produced initial dilation followed by vasoconstriction. Green and Stoner (1950) observed that intravenous injections of adenosine increased pulmonary artery pressure in cats. More recently Hyman et al. (1971) reported that intra-lobar infusion of adenosine had no effect in 2 dogs but Wiklund et al. (1987) reported that adenosine (approximately 1 to 100 μM) enhanced basal tone and contractile responses to transmural nerve stimulation and noradrenaline in isolated strips of guinea pig pulmonary artery. Furthermore Biaggioni et al. (1989) found that intravenous infusions of adenosine caused pulmonary vasoconstriction in unanaesthetised sheep. These changes were associated with a slight fall in cardiac output so they are of doubtful relevance to humans.

In contrast to most of the previous observations Mentzer et al. (1975) showed that adenosine infusion in the dog caused pulmonary vasodilation during normoxia and prevented the pulmonary pressor response to hypoxia. The latter effect has also been shown recently in isolated perfused rat lungs (Jolin et al., 1989). The cause of the discrepancies in these observations is not clear but there are probably species differences. In addition adenosine might exert contrasting effects at different concentrations or at different sites in the pulmonary artery wall. A further possibility is suggested by the recent observations in the cat by Neely et al. (1989) that at low pulmonary vascular tone adenosine was vasoconstrictor whereas when pulmonary vascular tone was increased it was vasodilator.

Recently, several studies have shown a fall in pulmonary vascular resistance of approximately 30% during adenosine infusion in anaesthetised patients (see Table 7.5), and Barnes et al. (1988) reported that adenosine relaxed pre-constricted human pulmonary arteries in vitro. Although patients with pulmonary hypertension were not studied in the present work, the results suggest that the effects of adenosine on the pulmonary circulation in such patients merit investigation. Gaba et al. (1986)

reported that adenosine did not alter pulmonary vascular resistance in patients with chronic obstructive pulmonary disease. However the highest dose of adenosine given was equivalent to only 27 μg/kg/min, compared with the mean lowest dose in the present study of 57μg/kg/min, and all of their patients were also treated with the potent adenosine antagonist theophylline.

7.4.2 - EFFECTS OF ADENOSINE ON HEART RATE AND BLOOD PRESSURE

This study confirmed the findings discussed in earlier chapters that adenosine infusion in conscious subjects increases heart rate but does not affect mean arterial blood pressure, although it may increase pulse pressure.

The mechanisms of the increase in heart rate are not clear. A response to symptoms may contribute. Some but not all workers have shown sympathetic activation during adenosine infusion in conscious subjects (Biaggioni et al., 1986; Biaggioni et al., 1987; Fuller et al., 1987; Conradson et al., 1987; Edlund et al., 1990). However Conradson et al. (1987) suggested that the increase in heart rate is mediated predominantly by reduced cardiac vagal tone. This is supported by the present finding that the increase was not modulated by treatment with a ß-blocker. However this finding contrasts with the observation that ß-blockade did seem to reduce the increase in heart rate caused by adenosine in the study discussed in Chapter 4, suggesting that at least in that study sympathetic activation contributed to the heart rate changes.

A major component of the increase may be secondary to the augmentation of ventilation, mediated in a similar way to the increase in heart rate caused by hypoxic stimulation of the carotid body in dogs (Daly & Scott, 1958) or systemic hypoxia in man (Kimura et al., 1988). Consistent with this suggestion is the finding that the increase in heart rate correlated with the increase in minute ventilation, but not with the changes in systemic or pulmonary vascular resistance, which might have been

expected to cause reflex effects. Furthermore, injection of adenosine in the aortic arch proximal to the carotid circulation has been shown to cause an initial increase in heart rate and systolic blood pressure before the onset of peripheral vasodilation, whereas more distal injection does not (Biaggioni et al., 1987). Against this explanation is the finding in the present study that at the lowest dose causing a significant increase in ventilation heart rate was unchanged.

That a smaller increase in heart rate has been observed in anaesthetised subjects may be partly due to pharmacological (Zimpfer et al., 1981) or mechanical attenuation of the ventilatory changes. However other factors such as a greater direct negative chronotropic action due to the larger doses of adenosine used or attenuation of baroreceptor effects cannot be excluded. The present results suggest that the bigger increase in heart rate in conscious subjects contributes substantially to the larger rise in cardiac index and consequently the unchanged mean blood pressure despite peripheral vasodilation.

As discussed in Chapter 1 adenosine can depress conduction in the atrioventricular node. Although a slight increase in mean PR interval and occasional first degree heart block have been observed with adenosine infusion during anaesthesia (Öwall et al., 1988a), no such changes were observed in the present study, where lower doses of adenosine were used.

7.4.3 - EFFECTS OF ADENOSINE ON LEFT VENTRICULAR FILLING PRESSURE

An unexpected finding in this study was an acute increase in left ventricular end-diastolic pressure and its indirect measure pulmonary capillary wedge pressure at the higher doses of adenosine.

Ischaemia might have caused these changes. Occasional cases of ischaemia have been observed during adenosine-induced hypotension (Öwall et al., 1988a).

However in those cases the hypotension would have reduced coronary perfusion pressure. Edlund et al. (1989) reported that adenosine infusion caused typical anginal pain in 6 patients with known ischaemic heart disease, with the development of associated ST segment depression suggestive of ischaemia on the ECG in 5. These workers suggested that the changes might be due to an adenosine-induced coronary steal, since they occurred with only minor increases in cardiac work, as judged by the small alterations in heart rate and unchanged blood pressure. However Belvedere et al. (1990) reported that during adenosine infusion at 140 μg/kg/min chest pain was equally common in normal volunteers (22/41) and patients with coronary disease (15/26). Furthermore whilst 8/26 patients developed "non-specific" ECG changes none showed changes diagnostic of ischaemia.

A coronary steal phenomenon has been described during adenosine infusion in dogs (Gallagher et al., 1980; Patterson & Kirk, 1983; Gewirtz et al., 1983) but may be more likely to occur when coronary perfusion pressure is reduced (Buffington et al., 1987). The increase in myocardial work suggested by the increase in the rate-pressure product in the present study might also have contributed to the development of ischaemia. However the change in end-diastolic pressure occurred equally in patients with and without coronary artery disease, with and without chest pain (discussed below) and, except in 2 patients, without ECG changes consistent with ischaemia.

The rise in end-diastolic pressure might represent the consequence of a negative inotropic effect, consistent with the known ability of adenosine to antagonise both the release of noradrenaline and the ß-receptor mediated responses to catecholamines in the myocardium as discussed in Chapter 1. This might also explain the further observation, albeit in small numbers, that the rise in end-diastolic pressure was smaller in patients investigated 30 to 45 h after stopping atenolol, a time when rebound ß-receptor hypersensitivity has been

demonstrated (Schwartz et al., 1981). However, it is difficult to implicate a negative inotropic effect when the rise in left ventricular end-diastolic pressure was associated with an increase in stroke output.

A further possible explanation is that adenosine might have reduced ventricular compliance by increasing turgor through its coronary dilator action (Watt et al., 1987c), as has been described in experimental animals (Vogel et al., 1982). Limitation of such an increase in turgor by hypotension and reduced coronary perfusion pressure could explain the apparent absence of an adenosine-induced increase in left ventricular filling pressure during anaesthesia.

7.4.4 - EFFECTS OF ADENOSINE ON RESPIRATION

In this study ventilation was first increased at a dose of adenosine that caused no haemodynamic changes apart from a fall in pulmonary vascular resistance. Wasserman et al. (1974) showed in dogs that an increase in cardiac output causes an increase in pulmonary ventilation, so-called "cardiodynamic hyperpnea". Such a mechanism might contribute to the ventilatory changes associated with adenosine infusion at the higher doses used in this thesis but could not explain the changes seen at the lowest dose in this study, when cardiac output was unchanged. Furthermore in the study described in Chapter 4 the site-dependent changes in heart rate, a major component of the changes in cardiac output caused by adenosine, and ventilation during intra-aortic adenosine infusion were not parallel.

The increase in left ventricular filling pressure might also contribute to changes in ventilation via stimulation of stretch receptors in the left atrium or pulmonary circulation. That this mechanism is unlikely to be quantitatively important is indicated by the observations in Chapter 4 that adenosine infusion into the ascending aorta immediately proximal to the carotid circulation, which is unlikely to have changed left

ventricular filling pressure, caused a similarly large increase in ventilation.

The changes in arterial gas tensions and pH are likely to have antagonised the effects of adenosine on ventilation.

7.4.5 - EFFECTS OF ADENOSINE ON OXYGEN CONSUMPTION

The increase in oxygen consumption following adenosine infusion is surprising. During adenosine infusion in anaesthetised subjects slight falls in whole body oxygen consumption have been observed in some studies, possibly due to shunting of blood away from metabolically active tissues or inhibition of metabolic demands (Sollevi et al., 1984b; Lagerkranser et al., 1984). In the present study such effects might have offset the increased oxygen consumption demanded by the greater cardiac and respiratory work during adenosine infusion. A rebound phenomenon after withdrawal of such mechanisms may have contributed to the increase in oxygen consumption following the infusion.

7.4.6 - SYMPTOMS CAUSED BY ADENOSINE

Symptoms described in this study were similar to those reported in the studies described in earlier chapters. Sylvén et al. (1986) suggested that adenosine release during myocardial ischemia might mediate the symptom of angina. There is however evidence that adenosine may have a more generalised algogenic effect (Bleehen & Keele, 1977; Watt et al., 1987b; Sylvén et al., 1988). This is supported by the present observations that, although in some patients with coronary disease adenosine appeared to reproduce their typical pain, it also did so in some patients thought to have non-cardiac pain, while in others with coronary disease it produced a discomfort different from their angina. These observations differ from those of Crea et al. (1990) who reported that in 20 patients with angina who developed chest pain during adenosine infusion the pain was similar to their angina

in each case. The mechanism of adenosine-induced pain remains to be fully elucidated but the pain does not seem to be secondary to vasodilation in either the brachial or coronary arteries (Sylvén et al., 1988; Sylvén et al., 1989; Crea et al., 1990).

7.4.7 - INTERACTIONS OF PATIENTS' MEDICATION WITH ADENOSINE

Some patients were receiving a variety of drugs during this study and the studies described in Chapters 4 and 6 but, apart from ß-adrenoceptor antagonists, there is no clear evidence that these drugs affected the responses to adenosine. However the absence of an increase in systolic and reduction in diastolic blood pressure caused by adenosine, as have been found previously (Biagionni et al., 1986; this thesis Chapters 3 and 5), might have been due to effects of patients' medications.

Conradson et al. (1987) and Jonzon et al. (1989) found that ß-adrenoceptor antagonists such as atenolol had no significant effect on the changes in ventilation or simple haemodynamic variables produced by adenosine infusion. As discussed above the only interaction observed in this study was a possible modulation of the effect of adenosine on left ventricular filling pressure following withdrawal of ß-adrenoceptor antagonists. Why attenuation by ß-blockade of the heart rate changes was only seen in the study described in Chapter 4 is unexplained, but it might relate to the different site of infusion of adenosine used in that study.

Based on an uncontrolled study Ikram et al. (1973) suggested that at therapeutic doses diazepam can increase coronary blood flow in man. Furthermore Clanachan and Marshall (1980a) showed that diazepam potentiated the coronary vasodilator response to adenosine in dogs, but large doses (1 to 4 mg/kg) of diazepam were required. However there is evidence that at low micromolar concentrations diazepam can inhibit the cellular uptake of adenosine and potentiate its actions in a variety of

tissues including the heart (Clanachan & Marshall, 1980b; Kenakin, 1982; Mehta and Kulkarni, 1984; Ruiz et al., 1988) and it has been suggested that benzodiazepines may exert some of their therapeutic actions by potentiating the effects of endogenous adenosine (Phillis & O'Regan, 1988). Even at 0.1 μM diazepam caused at least 20% inhibition of adenosine uptake in rat brain synaptosomes (Phillis et al., 1980). Hopkins (1973) showed that such a degree of inhibition in guinea pig atria was enough to cause a 2-fold potentiation of the actions of adenosine.

During the present study the mean plasma diazepam concentration is likely to have been less than 1 μM (Hillestad et al., 1974; Gamble et al., 1975) but, since the concentration of diazepam in some tissues might have exceeded that in plasma (Friedman et al., 1986), tissue concentrations of diazepam might have been sufficient to influence the responses to adenosine, although this is uncertain. Diazepam has been shown by some workers to depress ventilation (Dalen et al., 1969) and the ventilatory responses to hypoxia (Lakshminarayan et al., 1976) and hypercapnia (Catchlove & Kafer, 1971) and cause minor haemodynamic changes (Dalen et al., 1969). However the symptoms and heart rate and ventilatory changes in this study were similar to those observed at similar doses of adenosine in healthy volunteers who had not received diazepam (see Chapter 3).

Merrill et al. (1982) reported that nifedipine inhibited the coronary vasodilator response to adenosine in anaesthetised dogs. The mechanism was uncertain. However in vitro studies have been unable to demonstrate antagonism of adenosine-induced vascular relaxation by concentrations of nifedipine at the lower end of the range encountered with usual therapeutic doses (Young & Merrill, 1983; Mustafa & Askar, 1986; Sorkin et al., 1985). Furthermore Rooney et al. (1989) reported that nifedipine had no effect on the dose-requirement or the associated systemic haemodynamic changes during adenosine-induced hypotension in anaesthetised dogs.

Nifedipine has been reported to antagonise the binding of the adenosine analogue R-PIA to A_1 receptors (Cheung et al, 1987; Hu et al., 1987), providing a possible basis for antagonism of some actions of adenosine. However nifedipine can also inhibit cellular uptake of adenosine, thereby potentiating its actions (Marangos et al., 1984; Hammond et al., 1985). Half-maximal effects for both interactions require nifedipine concentrations at least several-fold greater than are encountered with usual therapeutic doses in man, i.e. less than approximately 150 μg/l (0.4 μM) (Sorkin et al., 1985).

I know of no evidence of a direct interaction between adenosine and organic nitrates. Concentrations of isosorbide dinitrate sufficient to relax vascular smooth muscle do not cause changes in the concentration of cyclic AMP, which mediates many of the effects of adenosine (Matlib et al., 1985). Nitrates such as isosorbide dinitrate generate nitric oxide which stimulates the production of cyclic guanosine monophosphate (GMP) resulting in smooth muscle relaxation (Ignarro & Kadowitz, 1985). Since vascular smooth muscle relaxation by adenosine might also be mediated by cyclic GMP (see Chapter 1) the possibility exists of an interaction of the effects of adenosine and nitrates. Deussen et al. (1986) showed that increasing coronary flow with isosorbide dinitrate had no effect on the effluent perfusate concentration of adenosine in isolated perfused guinea pig hearts.

7.4.8 - IMPLICATIONS FOR THE POSSIBLE THERAPEUTIC USE OF ADENOSINE

This study shows that in conscious subjects adenosine is a pulmonary and systemic vasodilator as well as a respiratory stimulant. However side effects may limit its sustained use in conscious subjects to infusion rates ≤ 60 to 80 μg/kg/min. Nevertheless at such rates adenosine can reduce pulmonary vascular resistance without causing symptoms suggesting that its effects in patients with pulmonary hypertension merit further study.

CHAPTER 8 - THE EFFECTS OF ADENOSINE INFUSION IN PATIENTS WITH CHRONIC HYPOXIA

8.1 - INTRODUCTION

As discussed in previous chapters adenosine causes marked stimulation of respiration in normal subjects and in patients with ischaemic heart disease. There is fairly good evidence that this effect is mediated at least in part via an action in the carotid bodies. These observations raise two questions: firstly, whether the effects of adenosine are similar in patients with respiratory failure and, secondly, whether adenosine may be useful in treating that condition. In the study described in this chapter the effects of adenosine infusion were studied in patients with chronic hypoxia secondary to chronic obstructive airways disease.

8.2 - METHODS

8.2.1 - SUBJECTS

Six patients (1 female), aged 57 to 70 years (mean 65) were studied. All had been heavy smokers and suffered from chronic hypoxia secondary to chronic bronchitis and chronic obstructive airways disease. Although in most an arterial PO_2 of less than 60 mmHg (the cutoff point for the definition of oxygenation failure) had previously been documented when they were clinically stable, four had an arterial PO_2 slightly greater than this at the time of the study. All were stable at the time of the study. Patients with a history of asthma or previously documented reversibility of airways obstruction ($\geq$ 20% improvement in FEV_1 following a bronchodilator) were excluded since inhaled adenosine may cause bronchoconstriction in such patients (Cushley *et al.*, 1983). Details of the patients and their treatment are shown in Table 8.1.

Patients receiving theophylline preparations discontinued these at least 24 hours prior to the study

Table 8.1 - Patient characteristics.

Subject	Age (years)	Sex	Treatment	FEV_1 (l (%))	FVC (l (%))	FEV_1 /FVC	PaO_2 (mmHg)	$PaCO_2$ (mmHg)	pH
1	65	M	OTS D	0.73 (26)	2.09 (58)	0.35	61	51	7.37
2	65	F	SIB D	0.47 (23)	1.05 (41)	0.45	61	45	7.37
3	66	M	TSIB	0.46 (19)	1.35 (44)	0.34	64	40	7.39
4	57	M	SI PDiD	0.54 (18)	1.27 (34)	0.42	58	49	7.34
5	70	M	OTSI D	0.60 (24)	1.55 (44)	0.39	48	58	7.36
6	65	M	TSI DiD	1.04 (33)	3.26 (80)	0.32	64	36	7.45
Mean	65			0.64 (24)	1.76 (50)	0.38	59	46	7.38
SD	4			0.22 (5)	0.81 (17)	0.05	6	8	0.04

FEV_1, forced expiratory volume in 1s; %, % of predicted; FVC, forced vital capacity; PaO_2, arterial oxygen tension; $PaCO_2$, arterial carbon dioxide tension; pH, arterial pH; O, long term oxygen therapy; T, theophylline; S, inhaled salbutamol; I, inhaled ipratroprium bromide; B, inhaled beclomethasone; P, oral prednisolone; Di, digoxin; D, diuretics.

and blood was sampled from these patients at the time of the study for measurement of theophylline concentration. No restriction was placed on the use of inhaled drugs prior to the study. No subject needed to use them during the study.

8.2.2 - INFUSIONS

Adenosine was infused single blind via a plastic cannula in an ante-cubital vein. An infusion protocol similar to that described in Chapter 5 was used: stages of 5 min at each dose were used with recovery periods of at least 15 min between stages. Adenosine was given at an initial rate of 2.3 mg/min with increments, as tolerated, to 4.3, 8.5, 11.9 and 16.8 mg/min. A control infusion (0.9% sodium chloride) was also given, single blind, instead of

adenosine usually after at least one adenosine infusion stage causing definite subjective and objective changes. Subjects were aware that doses of adenosine would increase at each new infusion stage during the study but also that at any stage the control infusion might be substituted. Subjects were asked to report any symptoms every minute during and for 2 min after each stage. Prior to each new stage subjects were asked if they were willing to receive a further infusion. The protocol for the infusions and measurements made is illustrated in Fig. 8.1.

8.2.3 - MEASUREMENTS

Respiration was recorded using a RIP as described in Chapter 2. A 20-breath volume calibration was performed over a range of tidal volumes at the start of the study and a 10-breath check calibration at the end of the study. The two curves were combined and linear regression analysis using the method of least squares was performed to obtain the calibration factor used. At each time point respiratory and haemodynamic variables were averaged over at least 40 s.

The peak minute ventilation during each infusion and the simultaneous respiratory rate and tidal volume were used in the initial comparisons of the effects at different doses. For reasons discussed below a separate analysis of changes occurring during adenosine infusion at the maximum dose was performed. For this analysis ventilation during the final minute of the infusion was used for comparison.

A plastic cannula inserted under local anaesthetic into a radial artery was used for blood sampling and direct recording of arterial pressure with a Gould T4812AD transducer connected to a Physio-Control VSM1 monitor. Systolic and diastolic blood pressure were obtained from a mean of 5 to 10 beats. Heart rate was determined from an electrocardiogram which was monitored throughout. For heart rate and systolic and diastolic

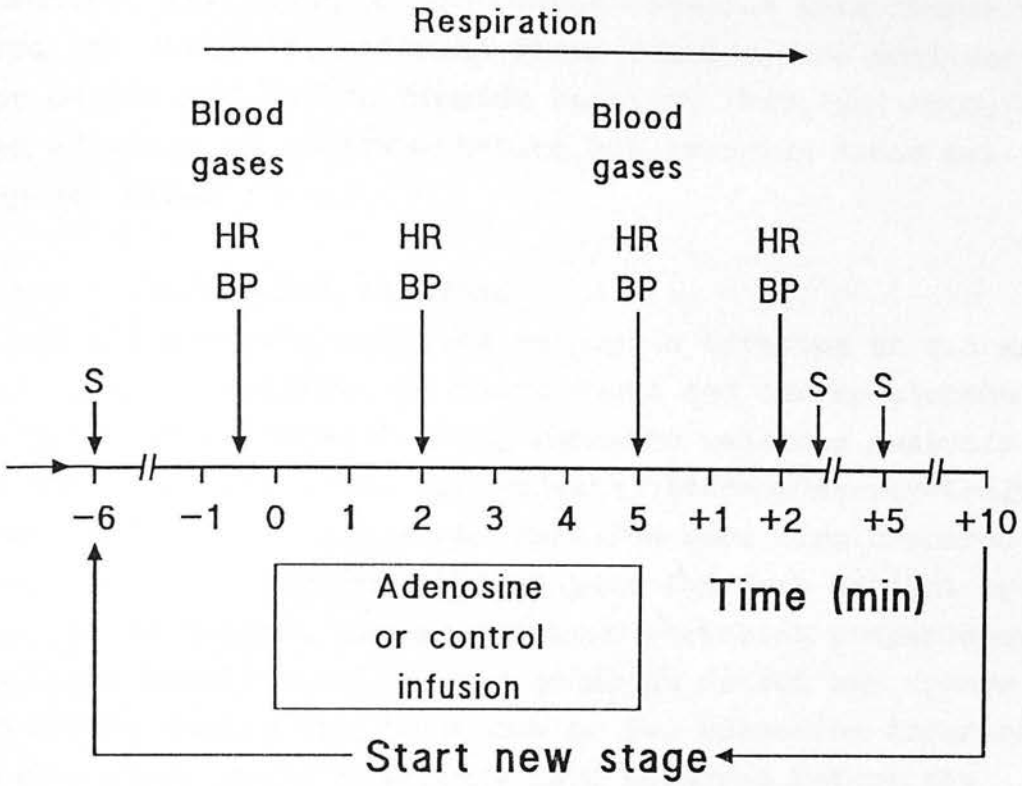

Fig. 8.1 - Protocol for infusions and measurements. HR, heart rate; BP, systemic blood pressure; blood gases, arterial blood gas tensions; S, spirometry.

blood pressures the values during infusion were taken as the mean of the 2 min and 5 min values. Mean blood pressure was calculated as:

$$\text{Mean BP} = \frac{\text{Systolic BP} + 2 \times \text{Diastolic BP}}{3} \qquad \text{(Eq. 8.1)}$$

Spirometry was performed in the posture maintained during the study using the same system as described in Chapter 5. Apart from the initial baseline test where 3 recordings were made only one recording was made at each time point to prevent fatigue and provocation of bronchospasm. For similar reasons every subject did not perform tests at 3 separate time points following each

infusion. The means of the values obtained were therefore used for analysis. Arterial blood samples were analysed for oxygen and carbon dioxide tensions (PaO_2 and $PaCO_2$) and pH using an Instrumentation Laboratories Blood Gas Manager Model 1312.

8.2.4 - STATISTICAL ANALYSIS

Since all patients received adenosine infusion at 2.3 and 4.3 mg/min, variables at those doses and during placebo infusion were compared using repeated measures analysis of variance and, where appropriate, Student-Newman-Keuls test. Values during placebo infusion were also compared with values during the maximum dose for each patient by the paired t-test. For spirometric variables comparisons included baseline values, in order to detect any change occurring during the first one or two adenosine infusion stages which would therefore have occurred before the placebo stage. Log transformation of blood pressures was performed before analysis. Except where indicated n = 6.

8.3 - RESULTS

In the 4 patients who had discontinued treatment with a theophylline preparation the serum theophylline concentration ranged from 0.9 to 2.0 mg/l. The maximum dose of adenosine infused was limited in all patients by symptoms and ranged from 4.3 to 16.8 mg/min (73 to 248 μg/kg/min; mean: 137 μg/kg/min). In 2 patients the final infusion was stopped after less than 5 min at the patients request because of symptoms.

8.3.1 - BASELINE VALUES

Baseline heart rate before the placebo infusion was significantly higher than the values before adenosine infusion at 2.3 and 4.3 mg/min ($P < 0.025$), but was not different from the value before the maximum dose. There were no other differences in baseline values (Table 8.2).

Table 8.2 - Baseline data for haemodynamic variables, minute ventilation, blood gas tensions and pH.

	Placebo	Adenosine Infusion		
		2.3 mg/min	4.3 mg/min	Maximum
Heart rate (beats/min)	87 (7)	82 (5)	84 (6)	85 (6)
Systolic blood pressure (mmHg)	133 (20)	138 (21)	132 (14)	133 (14)
Diastolic blood pressure (mmHg)	65 (13)	66 (12)	65 (10)	65 (10)
Minute ventilation (l/min)	7.5 (2.9)	7.3 (2.1)	6.9 (1.6)	7.9 (3.1)
PaO_2 (mmHg)	59* (6)	60* (7)	61* (5)	60 (6)
$PaCO_2$ (mmHg)	46* (10)	46* (9)	46* (9)	47 (9)
pH	7.38* (0.04)	7.39* (0.04)	7.38* (0.04)	7.38 (0.04)

Data are mean (SD). *n = 5, otherwise n = 6. Abbreviations as in Table 8.1.

8.3.2 - CHANGES IN VENTILATION AND BLOOD GAS TENSIONS

The changes in tidal volume and respiratory rate at peak minute ventilation at each infusion rate were not statistically significant. Nevertheless initial analysis showed that, compared with placebo, peak minute ventilation increased by 20% during adenosine infusion at the maximum dose ($P < 0.05$) (Table 8.3). However comparison of volume calibration curves for the RIP performed at the beginning and end of the study in the 5 subjects for whom both were available showed that there were differences between the slopes of more than 10% for 3 subjects. Since such changes may have contributed to the apparent increase in minute ventilation, a separate comparison was performed of minute ventilation at baseline immediately before adenosine infusion at the maximum dose with the value during the final minute of that infusion. The increase in minute ventilation from 7.9 (SD: 3.1) l to 8.8 (SD: 3.3) l was not significant.

Four patients (Numbers 1,4,5,6) received at least one

Table 8.3 - Haemodynamic and ventilatory changes during adenosine infusion.

	Placebo	Adenosine infusion		
		2.3 mg/min	4.3 mg/min	Maximum
Heart rate (beats/min)	81 (7)	84 (5)	86 (8)	90 (20)
Systolic blood pressure (mmHg)	130 (19)	138 (18)	140*** (20)	156** (27)
Diastolic blood pressure (mmHg)	62 (12)	66 (12)	72 (23)	75 (23)
Mean blood pressure (mmHg)	85 (14)	90 (14)	95* (22)	101* (24)
Peak minute ventilation (l/min)	8.0 (2.6)	8.3 (2.1)	8.2 (1.7)	9.5* (2.6)
Tidal volume (l)#	0.36 (0.10)	0.37 (0.06)	0.37 (0.07)	0.43 (0.15)
Respiratory rate (breaths/min)#	22 (6)	22 (3)	23 (5)	23 (7)
PaO_2 (mmHg)	59 (6)	59 (8)	60 (8)	58 (8)
$PaCO_2$ (mmHg)	46 (9)	44 (10)	45 (9)	45 (7)
pH	7.39 (0.04)	7.39 (0.04)	7.40 (0.03)	7.39 (0.03)

Data are mean (SD). n = 6. For comparisons with data during placebo infusion: *P < 0.05; **P < 0.01; ***P < 0.001. #, measured at time of peak minute ventilation. Other abbreviations as in Table 8.1.

dose of adenosine without symptoms. At the highest such dose the peak minute ventilation, 9.0 (SD: 2.2) l/min, was not significantly different from the value during placebo, 8.6 (SD: 2.7) l/min.

For the group there were no significant changes in PaO_2, $PaCO_2$ or pH during adenosine infusion at any dose (Table 8.3). Furthermore comparison of blood gas tensions during the final minute of adenosine infusion at the maximum dose with the baseline value for that infusion showed no significant change (Table 8.4). However individual responses were variable and 4 patients showed an increase in both tidal volume and minute ventilation and a slight improvement in blood gas tensions (Table 8.4).

Table 8.4 - Changes during the final minute of adenosine infusion at the maximum dose.

Subject	Dose (μg/kg /min)	Changes									
		HR (beats /min)	SBP (mmHg)	DBP (mmHg)	MBP (mmHg)	FRC (l)	V_T (l)	V (l/min)	PaO_2 (mmHg)	$PaCO_2$ (mmHg)	pH (units)
1	175	-13	21	1	9	0.34	0.09	2.0	1	-2	0.03
2	97	10	20	13	16	0.22	0.04	1.2	5	-3	0.02
3	94	60	69	47	63	0.41	-0.09	0.9	-7	1	-0.01
4	73	6	14	6	8	0.34	0.08	1.9	2	-7	0.02
5	131	8	23	6	10	0.22	0.01	1.2	1	-3	0.03
6	248	-9	20	3	12	0.77	-0.14	-1.6	-10	2	-0.02
Mean	137	10	28	13	20	0.38	0.00	0.9	-1	-2	0.01
SD	65	26	20	17	21	0.20	0.09	1.3	6	3	0.02

Changes are calculated with respect to the baseline values before infusion at the maximum rate. Positive values represent an increase, negative values a decrease. HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure; FRC, functional residual capacity; V_T, tidal volume; V, minute ventilation. Other abbreviations as in Table 8.1.

In all patients a shift upwards in the baseline of the respiratory trace occurred during adenosine infusion suggesting that they were breathing at a higher FRC. During adenosine infusion at the maximum dose, where this shift was most marked, the mean increase in FRC was 0.28 (SD: 0.14) l which was significantly different from the mean change of -0.02 (SD: 0.08) l during placebo infusion ($P < 0.05$) (Fig. 8.2). Inspection of the respiratory traces showed that in all patients the baseline shift occurred predominantly in the compartment which contributed most to tidal volume. In 4 patients the shift

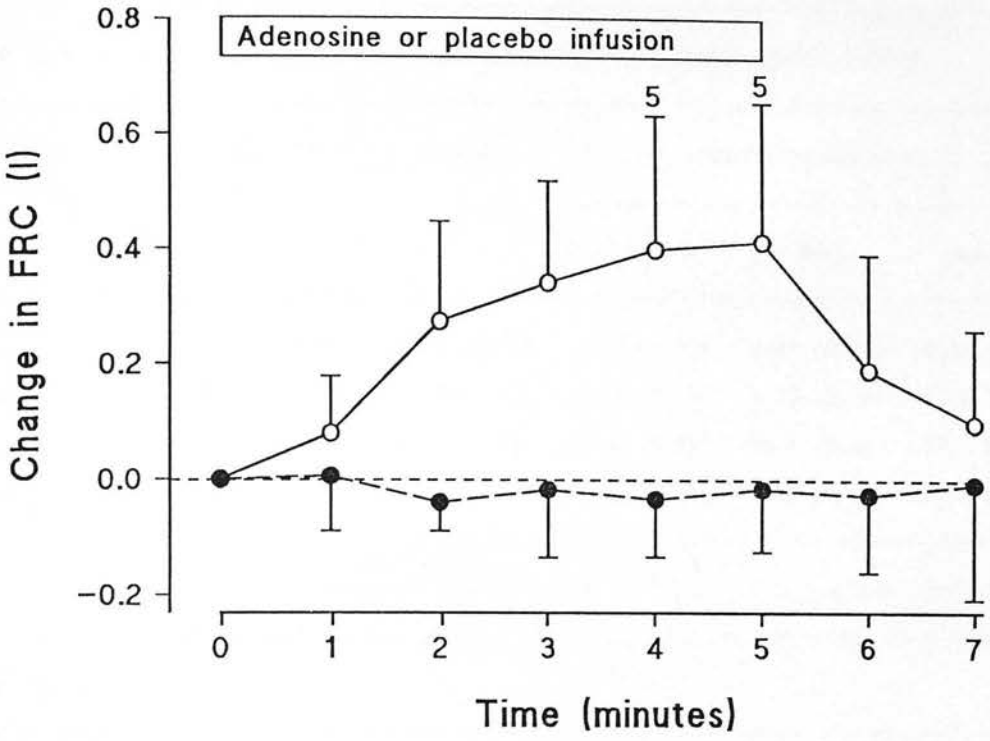

Fig. 8.2 - Changes in functional residual capacity (FRC) during adenosine and placebo infusions. n = 6 except where shown.

was seen only or predominantly in the abdominal compartment, and in 2 only or predominantly in the thoracic compartment. Of the 4 patients who received at least one dose of adenosine which did not cause symptoms, 2 (numbers 4,6) showed an obvious increase in FRC at the highest such dose. These changes were 0.24 and 0.36 l, compared with -0.01 and -0.02 l respectively during placebo.

The 2 patients who showed the largest increase in FRC (numbers 3,6) both showed reduced tidal volume and worsened blood gas tensions. One also showed a decrease in minute ventilation (Table 8.4).

8.3.3 - CHANGES IN SPIROMETRY

There were no significant changes in FEV_1, FVC, FEV_1/FVC

Table 8.5 - Spirometry in semi-recumbent position.

	Baseline	Placebo	Adenosine Infusion		
			2.3 mg/min	4.3 mg/min	Maximum
FEV_1 (l)	0.45* (0.15)	0.40 (0.17)	0.40 (0.13)	0.39 (0.16)	0.37 (0.16)
FVC (l)	1.28* (0.51)	1.13 (0.57)	1.11 (0.47)	1.17 (0.59)	1.04 (0.53)
FEV_1/FVC (%)	36.4* (6.6)	36.6 (4.9)	37.6 (6.3)	34.9 (4.6)	37.1 (5.5)
$FEF_{25-75\%}$ (l/s)	0.23** (0.07)	0.15 (0.06)	0.17 (0.05)	0.16 (0.06)	0.15 (0.05)

Data are mean (SD). n = 6 except where shown. *n=5; **n=4. FEV_1, forced expiratory volume in 1s; FVC, forced vital capacity; $FEF_{25-75\%}$, forced expiratory flow rate at 25-75% of FVC.

or $FEF_{25-75\%}$ following adenosine infusion at any dose (Table 8.5).

8.3.4 - CHANGES IN HEART RATE AND BLOOD PRESSURE

For the group as a whole heart rate showed no significant change during adenosine infusion (Table 8.3) but individual responses were variable. One patient showed a marked increase in heart rate at the maximum dose, by 60 beats/min during the infusion, but the two patients who received the highest doses of adenosine showed decreases in heart rate at the maximum dose (Table 8.4).

Systolic blood pressure increased significantly during adenosine infusion at 4.3 mg/min ($P < 0.001$) and at the maximum dose ($P < 0.01$) (Table 8.3). Changes in diastolic blood pressure were not significant but calculated mean blood pressure increased significantly during adenosine infusion at 4.3 mg/min ($P < 0.05$) and at the maximum dose ($P < 0.05$).

8.3.5 - SYMPTOMS CAUSED BY ADENOSINE

Symptoms experienced during adenosine infusion were

similar to those reported by normal subjects and patients with ischaemic heart disease. Dyspnoea occurred in 6 patients, central chest discomfort and headache each in 3 patients, flushing in 2 patients and throat discomfort in 1 patient. All symptoms resolved within 1 to 2 minutes of discontinuing the infusion. The dose at which any new symptom was first reported ranged from 2.3 to 16.8 mg/min (35 to 248 μg/kg/min).

8.4 - DISCUSSION

8.4.1 - CHANGES IN VENTILATION

This study investigated the effects of symptom-limited adenosine infusion in patients with chronic hypoxia secondary to chronic obstructive airways disease. Adenosine caused variable responses with only modest improvements at best in minute ventilation.

As discussed in Chapter 2 respiratory inductance plethysmography has been evaluated in patients with chronic obstructive airways disease and been shown to provide estimates of tidal volume within 10% of values obtained simultaneously using a spirometer, provided that calibration is carefully performed (Gonzalez et al., 1984; Dadzie et al., 1988). Even were the errors greater than this in the present study they are unlikely to have been large enough to invalidate the above conclusion concerning the magnitude of the ventilatory stimulation in these patients. Furthermore this conclusion is supported by the observed changes in the blood gas tensions.

The results of this study contrast with the previous findings in subjects without pulmonary disease, where maximal doses of adenosine caused a mean increase of approximately 80 to >100% in minute ventilation and falls in $PETCO_2$ of approximately 8 to 10 mmHg.

The smaller ventilatory stimulation by adenosine in the present patients might have been due to decreased sensitivity of the peripheral chemoreceptors. Since

endogenous adenosine concentrations have been shown to increase in a variety of tissues in various species during hypoxia, as discussed in Chapter 6, the endogenous adenosine concentrations might have already been increased in these patients. Whether this does occur in such patients is unknown, but increased plasma adenosine concentrations have been found in patients with sleep apnoea and severe nocturnal hypoxaemia (Findley et al., 1988). If a chronic increase in adenosine concentrations were present, the adenosine-dependent mechanisms might have already been substantially activated and down-regulation of adenosine receptors might have occurred.

While it is possible that a centrally-mediated respiratory depressant effect of adenosine opposing the peripherally-mediated stimulation of respiration contributed to the changes we observed, there is no evidence that such an effect should be greater in the present patients than in healthy volunteers.

It is possible that the adenosine-induced worsening of hypoxia in 2 patients was caused by increased ventilation/perfusion mismatching due to pulmonary vasodilation (Chapter 7, this thesis; Mentzer et al., 1975). However the fall in PaO_2 in these patients was associated with an increase in $PaCO_2$ and fall in tidal volume suggesting that reduced ventilation was responsible.

Theophylline is a competitive antagonist of the actions of adenosine at cell-surface receptors and at a concentration of approximately 10 μg/ml attenuates the cardiorespiratory effects of adenosine in man (Maxwell et al., 1987). In vitro studies have shown that half maximal effects of theophylline on responses to adenosine occur at concentrations ranging from approximately 1 to 10 μg/ml depending on the tissue and species studied (Daly, 1983). Therefore the concentrations of theophylline found in 4 patients in the present study might have caused weak antagonism of the effects of adenosine, but it seems

unlikely that this should have qualitatively affected the responses observed.

A further possible explanation of the absence of a marked respiratory stimulant effect of adenosine is that ventilation was limited by mechanical factors in these patients with severe chronic obstructive airways disease. It is possible that an acute increase in FRC contributed to this. In support of this suggestion is the finding that the apparent increase in FRC was greatest in two patients who showed a fall in tidal volume and worsening of blood gas tensions.

8.4.2 - CHANGES IN FUNCTIONAL RESIDUAL CAPACITY

The apparent change in FRC is unlikely to have been due to altered posture since its major component was from the compartment contributing most to tidal volume in each patient. It might have been due to an increase in intrathoracic blood volume (Sjöstrand, 1951) caused by pulmonary vasodilation. However a similar change observed in healthy volunteers (Chapter 5) developed in concert with increased ventilation suggesting that it is most likely a reflection of altered control of breathing. Furthermore an increase in FRC can be seen in spirograms obtained invasively during adenosine administration (Jonzon et al.,1989).

Although symptoms experienced may have been partly responsible for the change, an increase in FRC was also seen in some patients at doses of adenosine not causing symptoms. The time course of the change, with a rapid recovery following the infusion, in both healthy volunteers and patients, suggests that it was not due to airways obstruction (Cushley et al., 1983). Furthermore spirometry did not change in the present subjects or in the healthy volunteers (see Chapter 5). It is of interest that some, although not all, workers have reported that FRC increases during ventilatory stimulation by hypoxia in normal subjects (Garfinkel et al., 1978; Saunders et al., 1977; Kellogg & Mines, 1975; Cotes et al., 1977).

Similar changes in dogs or rats are abolished by carotid body blockade or denervation (Bouverot & Fitzgerald, 1969; Barer et al., 1978). Ventilatory stimulation by hypercapnia also increases FRC in normal subjects (Garfinkel et al., 1978) and in patients with chronic airflow limitation (Gribbin et al., 1983). In the latter case Garrard and Lane (1979) suggested that the increase in FRC is responsible for the diminished tidal volume response seen during hypercapnia in such patients.

8.4.3 - CHANGES IN HEART RATE AND BLOOD PRESSURE

In the present study adenosine produced variable changes in heart rate, with no significant change for the group, and significant increases in systolic and mean blood pressure. These responses differ from those observed in normal volunteers, in whom heart rate is consistently increased by maximal infusion rates of adenosine infusion and mean blood pressure usually remains constant due to a fall in diastolic pressure offsetting a rise in systolic blood pressure as discussed in Chapter 3.

As discussed in Chapter 7 reflexes secondary to peripheral chemoreceptor stimulation may contribute substantially to the haemodynamic effects of adenosine infusion. Studies in dogs have shown that, compared with animals in whom ventilation is mechanically restrained, the haemodynamic effects of hypoxic stimulation of the carotid bodies are opposite in direction in animals in whom ventilation is allowed to increase (Daly & Scott, 1963). The haemodynamic effects in the latter group probably occur as a reflex secondary to stimulation of pulmonary stretch receptors and reduced arterial PCO_2. The different effects of adenosine on ventilation and $PaCO_2$ in the present subjects compared with normal volunteers might therefore partly explain the differences in haemodynamic response.

The fall in heart rate seen in the patients who received the highest doses of adenosine might be due to the well-recognised direct negative chronotropic effect

of adenosine (see Chapter 1), unopposed by reflexes which in healthy volunteers cause heart rate to increase. A contribution of a baroreceptor reflex secondary to the increased blood pressure cannot however be excluded.

Blood pressure increased even in patients in whom heart rate fell indicating that either stroke volume or peripheral vascular resistance increased. Myocardial contractility is unlikely to have increased since in vitro studies have shown that adenosine antagonises the cardiac effects of catecholamines, as discussed in Chapter 1, and exerts little direct positive inotropic effect (Brückner, 1985). In most tissues the direct arterial effect of adenosine is vasodilation. However reflex arterial constriction increasing systemic vascular resistance or venoconstriction increasing venous return to the heart might have been responsible for the increase in blood pressure observed.

8.4.4 - IMPLICATIONS FOR THE POSSIBLE THERAPEUTIC USE OF ADENOSINE

The inter-individual variability in ventilatory and haemodynamic responses observed in the present study suggests that further work is required to identify which patient subgroups, if any, are most likely to derive benefit from the use of adenosine as a ventilatory stimulant. Adenosine might be more efficacious in patients with less mechanical limitation of breathing than those in the present study, e.g. patients with drug-induced respiratory depression, although there are as yet no data to support this speculation. Further studies will need to examine whether and to what extent the symptoms produced by adenosine contribute to the ventilatory changes seen and the degree to which such symptoms might limit the compound's possible therapeutic use. In any further studies caution should be used in interpreting indices of ventilatory drive which may be affected by changes in FRC, eg. mouth occlusion pressure (Fitzgerald et al., 1976).

CHAPTER 9 - SUMMARY

1. Diverse biological effects of the nucleoside adenosine have been described. This thesis takes as its starting point the observations of Watt and Routledge (1985) that intravenous bolus injections of adenosine cause transient stimulation of respiration in man. It discusses the results of studies undertaken to further characterise the respiratory and concomitant cardiovascular effects of adenosine in man and in so doing to answer some of the questions raised by the previous observations. In all studies respiration was measured non-invasively using either a respiration transducer (Lectromed, Type 4320) or a respiratory inductance plethysmograph (Respitrace).

2. To determine whether the respiratory stimulation by adenosine is sustained during continuous infusion, whether it is caused directly by adenosine or one of its metabolites and whether it is secondary to hypotension, due to the known vasodilator effect of adenosine, the cardiorespiratory effects of symptom-limited intravenous infusions of adenosine and its deaminated metabolite, inosine, administered in random order, single-blind, were compared in 6 healthy volunteers.

3. Adenosine infusion at rates of 6.1 mg/min and above increased mean minute ventilation (by 191% at the maximum dose, 13.5 (SD: 5.7) mg/min), principally due to an increase in tidal volume, and reduced end-tidal PCO_2 ($PETCO_2$) (from 38 (SD: 3) mmHg to 27 (SD: 5) mmHg at the maximum dose). Mean inspiratory flow rate increased and expiratory duration decreased, by 179% and 23% respectively at the maximum dose, but there was no change in inspiratory duration. Adenosine also increased heart rate by 57% and systolic blood pressure from 120 (SD: 10) mmHg to 134 (SD: 15) mmHg at the maximum dose, without a significant change in diastolic blood pressure.

4. Infusion of inosine at dose rates up to 16.8 mg/min produced no pharmacological effects.

5. This study shows that infusion of adenosine produces sustained respiratory stimulation in man which does not depend on prior conversion of adenosine to inosine or related metabolites and is not secondary to systemic hypotension.

6. Since in animals adenosine has been shown to stimulate the peripheral chemoreceptors, the effects of infusion of adenosine at different sites in the aorta were investigated in 12 patients undergoing cardiac catheterisation for investigation of chest pain, to determine whether adenosine might stimulate respiration via an action in the carotid bodies in man.

7. Respiration was stimulated when adenosine was infused proximal to the carotid circulation, but not when it was infused distal to the head and neck vessels. Since previous studies in animals suggest that respiratory stimulation by adenosine is unlikely to be mediated within the central nervous system, these results support the hypothesis that adenosine-induced respiratory stimulation in man is mediated in the carotid body.

8. In order to determine whether the cardiorespiratory effects of adenosine are mediated by cell-surface adenosine receptors, the effects of aminophylline, a competitive antagonist of adenosine at such receptors, on the responses to symptom-limited intravenous infusion of adenosine were investigated in 10 healthy volunteers.

9. Aminophylline (6 mg/kg infused over 10 min at the start of the study) alone increased heart rate by 13% and minute ventilation by 25%, but reduced the symptoms and antagonised the increases in heart rate, systolic blood pressure and minute ventilation and the reduction in

$PETCO_2$ caused by adenosine. This suggests that such effects of adenosine are mediated by stimulation of cell-surface receptors.

10. There was no significant adenosine-induced change in FEV_1, FVC, FEV_1/FVC or $FEF_{25-75\%}$ suggesting that the adenosine-induced changes in respiration are unlikely to be secondary to bronchoconstriction. Adenosine caused a significant increase in functional residual capacity (FRC) which developed in concert with the changes in minute ventilation suggesting that it was due to altered control of breathing.

11. The peripheral venous plasma adenosine concentration was measured, after conversion of adenosine to $1,N^6$-ethenoadenosine, using high performance liquid chromatography with fluorometric detection. A significant change during adenosine infusion was detectable only at the higher maximum dose possible after aminophylline, consistent with the very short half-life of adenosine (less than 10 s) as previously demonstrated by others <u>in vitro</u>. This shows that a change in the peripheral venous concentration of adenosine cannot be used as an index of a change at sites much more proximal in the circulation.

12. Whether the respiratory stimulant effect of adenosine occurs at concentrations likely to be achieved physiologically was investigated in a further 7 patients undergoing cardiac catheterisation, in whom it was possible to sample blood via a catheter from a site close to the putative site of action in the carotid bodies.

13. During symptom-limited intravenous infusion of adenosine (mean maximum dose per minute: 130 μg/kg/min) mean minute ventilation increased by 98% while mean plasma adenosine concentration in the aortic arch increased from 0.07 (range: 0.04 to 0.12) μM to 1.2 (range: 0.4 to 2.0) μM. In three patients ventilation

first increased without a detectable increase in aortic adenosine concentration, suggesting a possible intra-pulmonary effect of adenosine, although increased concentrations were apparent at higher doses.

14. Previous work suggests that micromolar concentrations of adenosine are probably achieved in tissues during hypoxia, secondary to increased hydrolysis of adenine nucleotides. This study shows that at such concentrations adenosine stimulates respiration. These results are consistent with the suggestion that endogenous adenosine release may mediate or modulate the ventilatory response to hypoxia. A possible intra-pulmonary effect of adenosine merits further study.

15. Since the cardiovascular and respiratory systems are intimately related and since adenosine has been shown to have various effects within the cardiovascular system, including dilation of most vascular beds, which may have therapeutic application, the acute haemodynamic effects of symptom-limited intravenous infusion of adenosine were studied in 16 patients (7 with coronary artery disease, 9 with normal coronary arteries) undergoing cardiac catheterisation for investigation of chest pain. Pressures were measured via fluid-filled catheters and cardiac output by thermodilution using a balloon-tipped (Swan-Ganz) pulmonary artery catheter.

16. At the lowest dose used (4.3 mg/min) adenosine increased minute ventilation by 44% (n = 11) and reduced pulmonary vascular resistance by 20% without causing other significant haemodynamic changes.

17. At the maximum dose (8.5 (SD: 2.3) mg/min) adenosine reduced pulmonary and systemic vascular resistance, by 38% and 34% respectively, and increased heart rate by 34%, stroke index by 12% and cardiac index by 52%. Systemic blood pressure and right atrial pressure did not

change. Unexpectedly, adenosine increased left ventricular end-diastolic pressure, from 5 (SD: 6) mmHg to 14 (SD: 10) mmHg (n = 8), pulmonary capillary wedge pressure, from 3 (SD: 2) mmHg to 10 (SD: 5) mmHg (n = 16) and consequently mean pulmonary artery pressure, from 10 (SD: 2) mmHg to 16 (SD: 5) mmHg. Minute ventilation increased by 84% (n = 11), resulting in hypocapnia ($PaCO_2$: 31 (SD: 3) mmHg. n = 8) and alkalosis (arterial pH: 7.46 (SD: 0.02). n = 8). Oxygen consumption was unchanged during the infusion but increased by 21% 5 min post infusion. All effects were similar in patients with and without coronary artery disease.

18. Adenosine therefore causes pulmonary and systemic vasodilation and increased cardiac output in addition to respiratory stimulation. Symptoms and the increase in left ventricular end-diastolic pressure which occurred at the higher doses, may limit the use of adenosine as a systemic vasodilator or respiratory stimulant in conscious subjects. However at low infusion rates ($\leq$ 60 to 80 μg/kg/min) adenosine causes selective pulmonary vasodilation which merits further study.

19. Since adenosine might be useful in treating respiratory failure the effects of symptom-limited intravenous infusion of adenosine were studied in 6 patients with chronic hypoxia secondary to severe chronic obstructive airways disease.

20. The ventilatory responses to adenosine were variable. During maximum tolerated infusion rates 4 patients showed an increase in minute ventilation ranging from 1.2 to 2.0 l/min with a slight improvement in arterial blood gas tensions, while 2 patients showed a fall in tidal volume (of 0.09 and 0.14 l) and arterial PO_2 (of 7 and 10 mmHg respectively). All patients showed an increase in FRC ranging from 0.2 to 0.8 l, which did not seem to be due to bronchoconstriction and probably resulted from altered

control of breathing. The increase in FRC was greatest in the 2 patients who showed a fall in arterial PO_2. Changes in heart rate were variable but all patients showed an increase in mean blood pressure ranging from 9 to 52 mmHg (mean: 18 mmHg).

21. The magnitude of the effects of adenosine on ventilation contrasts with the prominent stimulation of respiration observed in healthy volunteers. Mechanical limitation of breathing, possibly exacerbated acutely by the increase in FRC, was probably at least partly responsible for the smaller and variable responses, but down-regulation of adenosine receptors in these chronically hypoxic patients cannot be excluded. The improvement in ventilation in some patients suggests that further evaluation of adenosine as a respiratory stimulant is indicated.

22. In all studies the higher doses of adenosine caused symptoms including: chest and epigastric discomfort, dyspnoea, flushing and paraesthesiae. Although significant group changes in minute ventilation were not observed in the absence of symptoms, a dissociation between the occurrence of chest and epigastric discomfort and respiratory stimulation was seen when adenosine was infused at different sites in the aorta. This suggests that the respiratory changes are not just a response to the symptoms caused by adenosine. Further work will be necessary to clarify the relationships between symptoms and other effects caused by adenosine and the extent to which such symptoms may limit any therapeutic use of adenosine in conscious subjects.

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APPENDIX 1 - MAIN INDIVIDUAL DATA FOR CHAPTER 3

SUBJECTS AND DOSES

Subject	Sex	Maximum dose received (mg/min)	
		Adenosine	Inosine
1	M	16.8	23.4
2	F	8.5	16.8
3	M	11.9	23.4
4	M	8.5	23.4
5	M	23.4	23.4
6	M	11.9	16.8
7	M	(11.9)	(23.4)
8	M	(8.5)	
Mean (n = 6)		13.5	21.2
SD		5.7	3.4

ADENOSINE INFUSION

Subject	Base-line	Adenosine infusion rate (mg/min)*							
		3.1		4.3		6.1		8.5	Maximum
				Respiratory rate (breaths/min)					
1	13			12	12	13	12	14	16
2	13	13	17	23	21	19	39	17	17
3	17	11	11	11	9	14	15	15	19
4	15	17	15	15	11	14	15	16	19
5	9	12	9	9	9	9	10	8	9
6	13	13	10	13	11	11	13	15	18
Mean	13	13	12	14	12	13	17	14	16
SD	3	2	3	5	4	3	11	3	4
				Tidal volume (l)					
1	0.69			0.64	0.73	0.70	0.62	1.36	1.15
2	0.10	0.25	0.36	0.38	0.14	0.35	0.21	0.41	0.41
3	0.07	0.27	0.33	0.31	0.49	0.24	0.82	0.84	1.55
4	0.51	0.51	0.47	0.48	0.75	0.48	0.86	0.78	0.53
5	0.86	0.62	0.56	0.74	0.67	0.82	1.00	0.89	1.57
6	1.00	0.93	1.20	1.13	1.21	1.20	1.49	1.35	2.00
Mean	0.54	0.52	0.58	0.61	0.67	0.63	0.83	0.94	1.20
SD	0.39	0.28	0.36	0.30	0.35	0.35	0.42	0.36	0.63
				Minute ventilation (l/min)					
1	9.2			7.4	8.5	8.8	7.1	18.6	18.2
2	1.3	3.2	6.2	8.8	2.9	6.6	8.2	7.1	7.1
3	1.2	3.1	3.7	3.4	4.6	3.4	12.3	12.6	29.5
4	7.6	8.4	6.9	7.3	8.3	6.9	12.7	12.3	10.2
5	7.5	7.3	5.2	6.4	5.7	7.6	9.5	6.9	14.6
6	12.6	12.4	11.6	14.7	13.0	13.7	20.0	20.5	35.8
Mean	6.6	6.9	6.7	8.0	7.1	7.8	11.6	13.0	19.2
SD	4.5	3.9	3.0	3.7	3.6	3.4	4.6	5.7	11.2
				End-tidal PCO_2 (mmHg)					
1	37			39	34	31	36	32	25
2	34	29	32	31	28	26	26	25	25
3	41	37	39	39	38	37	31	31	22
4	40	39	40	39	40	38	36	35	36
5	34	36	35	35	36	35	34	34	26
6	40	39	40	38	38	38	36	35	28
Mean	38	36	37	37	36	34	33	32	27
SD	3	4	3	3	5	5	4	4	5

*Two columns for any dose represent data for the first and second minutes.

Subject	T_I (s)		T_E (s)		T_{TOT} (s)		T_I/T_{TOT}		V_T/T_I (l/min)	
	B	Max	B	Max	B	Max	B	Max	B	Max
1	2.0	1.6	2.6	2.2	4.5	3.8	0.43	0.43	21	43
2	1.0	1.0	3.8	2.5	4.7	3.5	0.20	0.28	6	25
3	1.1	1.3	2.4	1.8	3.5	3.2	0.32	0.43	4	70
4	1.7	1.2	2.4	2.0	4.0	3.1	0.41	0.37	19	28
5	2.2	2.4	4.7	4.1	6.9	6.5	0.32	0.37	23	40
6	2.1	1.6	2.6	1.8	4.8	3.4	0.45	0.48	28	76
Mean	1.7	1.5	3.1	2.4	4.8	3.9	0.36	0.39	17	47
SD	0.5	0.5	1.0	0.9	1.2	1.3	0.09	0.07	10	21

B, baseline; Max, adenosine infusion at the maximum dose.

Subject	Base-line	Adenosine infusion rate (mg/min)*							
		3.1		4.3		6.1		8.5	Maximum
				Heart rate (beats/min)					
1	75			72	73	77	77	100	120
2	69	71	76	76	97	84	114	103	103
3	73	66	72	67	87	73	81	85	100
4	64	68	64	63	66	66	86	71	93
5	57	60	58	65	59	61	58	66	109
6	63	63	68	65	67	70	86	90	105
Mean	67	66	68	68	75	72	84	86	105
SD	7	4	7	5	14	8	18	15	9
				Systolic blood pressure (mmHg)					
1	124			134	131	124	144	134	124
2	112	108	110	110	112	115	118	124	124
3	106	108	112	112	114	112	124	132	134
4	116	114	114	122	120	122	126	122	120
5	125	120	125	128	125	123	120	123	138
6	134	144	146	140	140	150	148	150	162
Mean	120	119	121	124	124	124	130	131	134
SD	10	15	15	12	11	13	13	11	15
				Diastolic blood pressure (mmHg)					
1	90			92	91	104	86	84	76
2	72	74	72	72	80	74	72	68	68
3	54	56	56	56	62	58	62	58	64
4	60	60	70	80	80	80	70	60	42
5	73	80	75	80	78	79	75	65	52
6	86	86	86	84	86	82	94	96	96
Mean	73	71	72	77	80	80	77	72	66
SD	14	13	11	12	10	15	12	15	19

*Two columns for any dose represent data for the first and second minutes.

Subjects	**FEV_1** (l)		**FVC** (l)		**PEFR** (l/min)	
	Pre	Post	Pre	Post	Pre	Post
4	3.54	3.44	4.46	4.48	636	613
5	4.68	4.26	5.86	5.74	603	541
6	4.45	4.13	5.63	5.55	726	650
7*	4.33	4.55	5.00	5.29	576	570
Mean	4.25	4.10	5.24	5.27	635	594
SD	0.50	0.47	0.63	0.56	65	48

*subject whose other data were not included because of a poor respiratory trace.
Pre, prior to adenosine; Post, following adenosine.

INOSINE INFUSION

Subject	Baseline	Inosine infusion rate (mg/min)*					
	0	3.1	4.3	6.1	8.5	11.9	16.8
				Respiratory rate (breaths/min)			
1	10		11	10	12	13	13
2	15	16	17	20	18	15	11
3	12	18	20	18	19	18	15
4	16	18	16	14	15	14	14
5	14	13	11	10	13	13	11
6	9	11	6	9	9	12	9
Mean	13	15	14	14	14	14	12
SD	3	3	5	5	4	2	2
				Tidal volume (l)			
1	0.83		0.85	0.59	0.81	0.57	0.57
2	0.17	0.10	0.18	0.15	0.15	0.13	0.33
3	0.12	0.07	0.08	0.12	0.21	0.24	0.16
4	0.52	0.43	0.58	0.63	0.58	0.59	0.51
5	0.78	0.65	0.89	0.63	0.57	0.66	0.57
6	1.11	0.98	0.98	0.91	0.92	0.91	1.04
Mean	0.59	0.45	0.59	0.51	0.54	0.52	0.53
SD	0.39	0.38	0.38	0.31	0.31	0.29	0.30
				Minute ventilation (l/min)			
1	8.4		9.1	6.1	9.5	7.2	7.2
2	2.6	1.6	3.1	3.0	2.7	1.9	3.7
3	1.5	1.2	1.6	2.2	4.1	4.4	2.4
4	8.1	7.7	9.3	9.0	8.6	8.0	7.2
5	10.7	8.6	9.8	6.3	7.6	8.8	6.2
6	10.0	10.8	6.2	8.3	8.5	10.5	9.6
Mean	6.9	6.0	6.5	5.8	6.8	6.8	6.1
SD	3.9	4.3	3.5	2.8	2.8	3.1	2.6
				End-tidal PCO_2 (mmHg)			
1	33		36	40	36	37	37
2	33	33	30	30	33	33	29
3	41	43	43	43	43	42	40
4	40	40	41	40	39	40	41
5	35	39	38	36	37	38	37
6	38	38	40	40	40	34	38
Mean	37	39	38	38	38	37	37
SD	3	4	4	4	4	4	4

*For clarity only the 2 minute value at each dose is shown.

Subject	T_I (s)		T_E (s)		T_{TOT} (s)		T_I/T_{TOT}		V_T/T_I (l/min)	
	B	16.8	B	16.8	B	16.8	B	16.8	B	16.8
1	2.2	1.9	3.7	2.9	5.9	4.8	0.37	0.39	23	18
2	1.1	1.1	2.8	4.2	3.9	5.3	0.28	0.20	10	19
3	0.8	1.9	4.1	2.1	4.8	4.0	0.16	0.48	10	5
4	1.4	1.4	2.5	2.8	3.9	4.3	0.36	0.33	23	21
5	1.4	1.4	3.0	4.1	4.4	5.5	0.32	0.25	34	24
6	2.4	2.6	4.2	3.9	6.7	6.6	0.36	0.40	28	24
Mean	1.5	1.7	3.4	3.3	4.9	5.1	0.31	0.34	21	19
SD	0.6	0.5	0.7	0.9	1.1	0.9	0.08	0.10	10	7

B, baseline; 16.8, inosine infusion at 16.8 mg/min.

Subject	Base-line	Inosine infusion rate (mg/min)*					
		3.1	4.3	6.1	8.5	11.9	16.8
				Heart rate (beats/min)			
1	80		77	80	80	80	80
2	67	71	73	71	75	70	70
3	73	78	78	76	75	76	84
4	63	59	62	65	64	65	68
5	58	57	58	55	59	65	63
6	61	58	60	60	57	58	64
Mean	67	65	68	68	68	69	72
SD	8	9	9	10	10	8	9
				Systolic blood pressure (mmHg)			
1	110		124	134	128	132	133
2	105	110	109	100	112	110	110
3	106	110	106	110	108	106	106
4	108	108	114	116	106	110	106
5	125	123	125	125	123	125	125
6	136	140	140	136	138	140	140
Mean	115	118	120	120	120	121	120
SD	13	14	13	14	13	14	15
				Diastolic blood pressure (mmHg)			
1	92		93	84	82	84	84
2	68	68	74	72	70	72	74
3	44	52	48	52	48	42	54
4	70	70	74	70	70	68	70
5	70	70	65	75	68	75	82
6	86	90	88	84	88	82	84
Mean	72	70	74	73	71	71	75
SD	17	14	16	12	14	15	12

*For clarity only the 2 minute value at each dose is shown.

Subjects	FEV_1 (l)		FVC (l)		PEFR (l/min)	
	Pre	Post	Pre	Post	Pre	Post
4	3.46	3.48	4.29	4.45	616	592
5	4.47	4.51	5.94	5.87	529	547
6	4.24	4.28	5.57	5.65	630	688
7**	4.32	4.49	5.00	5.21	512	523
Mean	4.12	4.19	5.20	5.30	572	588
SD	0.45	0.49	0.72	0.63	60	73

**subject whose other data were not included because of a poor respiratory trace.
Pre, prior to inosine; Post, following inosine.

APPENDIX 2 - MAIN INDIVIDUAL DATA FOR CHAPTER 4

Patient details are shown in Chapter 4.

Subject	Baseline	Site 1	Site 2	Site 3	Site 4	Site 5
			Respiratory rate (breaths/min)			
1	13	15	11	13	13	16
2	13	15	13	13	13	17
3	16	15	18	15		14
4	11	10	12	15	14	12
5	19	21	24	13	18	21
6	17	17	20	17	7	19
7	16	14	12	15	14	13
9	22	22	24	13	14	20
10	14	16	13	19	14	16
11	21	19	18	19	19	19
Mean	16	16	17	15	14	17
SD	4	4	5	2	3	3
			Tidal volume (l)			
1	0.31	0.57	0.64	0.65	0.61	0.54
2	0.44	0.54	0.40	0.38	0.23	0.42
3	0.39	0.63	0.55	0.47		0.33
4	0.55	1.25	0.84	0.56	0.41	1.55
5	0.41	0.77	1.44	0.69	0.38	1.03
6	0.29	0.37	0.25	0.37	0.25	0.27
7	0.14	0.38	0.53	0.68	0.55	1.07
9	0.27	0.35	0.31	0.25	0.15	0.22
10	0.54	0.63	0.62	0.37	0.54	0.55
11	0.17	0.22	0.38	0.21	0.18	0.22
Mean	0.35	0.57	0.60	0.46	0.38	0.62
SD	0.14	0.57	0.34	0.18	0.18	0.45
			Minute ventilation (l/min)			
1	3.9	8.4	7.2	8.1	8.0	8.8
2	5.6	8.1	5.4	5.0	2.9	7.2
3	6.1	9.1	9.9	7.2		4.8
4	5.9	12.6	9.9	8.4	5.6	18.2
5	7.8	16.2	35.1	9.1	6.8	21.7
6	5.0	6.1	5.1	6.3	1.7	5.1
7	2.3	5.3	6.2	10.2	7.4	14.1
9	5.8	7.7	7.3	3.2	2.1	4.4
10	7.2	9.9	8.3	6.9	7.6	8.8
11	3.6	4.3	6.9	3.9	3.4	4.1
Mean	5.3	8.8	10.1	6.8	5.1	9.7
SD	1.7	3.5	8.9	2.3	2.5	6.2
			Heart rate (beats/min)			
1	52	64	64	62	62	68
2	53	68	67	69	72	71
3	61	65	74	73	67	71
4	53	53	55	61	59	65
5	78	86	102	102	100	112
6	62	60	68	70	68	68
7	49	50	60	57	53	62
8	66	82	88	90	84	104
9	61	64	72	70	73	64
10	48	44	48	57	54	62
11	71	72	71	74	82	85
Mean	59	64	70	76	71	70
SD	9	13	15	17	14	14

Subject	Baseline	Site 1	Site 2	Site 3	Site 4	Site 5
	Right femoral artery blood pressure (systolic/diastolic; mmHg)					
7	122/64	116/57	121/60	116/57	120/60	124/62
8	140/76	140/75	155/83	160/84	150/78	158/84
9	115/83	136/96	135/90	110/78	100/73	124/86
11	117/55	130/60	136/66	130/62	150/75	144/70
Mean	123/70	131/72	137/75	129/70	130/72	138/76
SD	11/12	11/18	14/14	22/13	24/8	17/11

Subject	Pre-adenosine	Post-adenosine
	Intra-aortic blood pressure (systolic/diastolic (mean); mmHg)	
3	110/60 (80)	100/50 (64)
4	112/62 (84)	116/60 (76)
5	122/70 (92)	125/70 (96)
6	110/70 (88)	125/60 (90)
7	125/75 (96)	110/60 (80)
8	135/75 (104)	125/65 (92)
9	140/65 (92)	140/65 (98)
10	120/50 (80)	110/48 (76)
Mean	122/66 (89)	119/60 (84)
SD	11/8 (8)	12/7 (12)

APPENDIX 3 - MAIN INDIVIDUAL DATA FOR CHAPTER 5

Abbreviations as in Chapter 5, unless otherwise stated.

SUBJECTS

Subject	Age (years)	Sex	Weight (kg)	Maximum adenosine dose (mg/min)	
				Placebo leg	Aminophylline leg
1	33	M	70	11.9	16.8
2	23	M	84	16.8	16.8
3	36	M	55	11.9	16.8
4	43	F	57	11.9	11.9
5	55	M	85	11.9	16.8
6	26	M	59	8.5	11.9
7	27	M	89	16.8	16.8
8	27	M	71	16.8	23.4
9	33	M	63	8.5	16.8
10	25	M	75	8.5	8.5
Mean	33		71	12.3	15.6
SD	10		12	3.4	4.0

BASELINE VALUES

PLACEBO LEG

Subject	Baseline							
	Pre	Post	1	2	3	4	5	Final
				Heart rate (beats/min)				
1	51	55	57	57	54	54	54	55
2	68	72	71	75	73	69	65	64
3	61		65	67	63	63	58	61
4	63	65	67	64	69	71	68	76
5	66	67	78	75	79	81	80	78
6	63	68	59	69	68	57	68	68
7	79	69	66	75	73	63	62	71
8	55	57	60	62	60	60	64	54
9	80	74	73	68	76	80	80	80
10	62		63	66	66	66	60	60
Mean	65	66	66	68	68	66	66	67
SD	9	7	7	6	8	9	9	9
				Systolic blood pressure (mmHg)				
1	100	102	108	108	110	110	106	108
2	124	126	126	125	121	120	126	129
3	102		94	98	98	94	88	100
4	100	106	102	94	104	98	106	104
5	142	145	145	145	144	150	147	156
6	86	84	84	86	92	86	90	90
7	130		118	116	122	124	112	110
8	114	120	115	112	122	120	122	123
9	106	110	106	95	99	108	106	106
10	100		122	112	136	120	114	114
Mean:	110	113	112	109	115	113	112	114
SD:	17	19	17	17	17	18	17	18

Subject	Baseline							
	Pre	Post	1	2	3	4	5	Final
				Diastolic blood pressure (mmHg)				
1	56	60	55	59	58	54	56	62
2	64	66	66	64	65	71	67	74
3	60		54	60	54	56	56	60
4	56	52	54	60	54	54	64	65
5	78	84	92	91	78	87	81	94
6	52	54	57	54	61	54	54	54
7	58		62	54	70	69	60	64
8	50	50	56	58	56	57	50	56
9	68	66	66	60	70	71	74	74
10	56		64	54	54	60	64	64
Mean	60	62	63	61	62	63	63	67
SD	8	12	11	11	8	11	10	12
				Minute ventilation (l/min)				
1	5.8	5.7	6.4	5.8	6.6	5.5	5.9	5.0
2	8.6	9.3	8.1	9.2	8.5	8.1	9.0	8.7
3	4.7		4.5	3.9	4.5	4.6	3.8	3.9
4	3.4	3.8	4.0	3.1	3.5	3.4	3.3	3.5
5	5.1	5.8	7.1	6.7	7.4	8.7	9.2	7.0
6	5.0	3.6	5.0	3.6	3.9	4.2	3.7	3.7
7	6.9	8.0	6.4	7.3	7.4	7.4	6.0	6.4
8	6.2	6.2	6.5	6.1	5.3	6.1	5.1	5.2
9	5.6	5.0	5.2	4.7	5.0	6.2	6.2	6.2
10	8.3		8.4	9.0	9.8	9.5	9.0	9.0
Mean	6.0	5.9	6.2	6.0	6.2	6.3	6.1	5.9
SD	1.6	1.9	1.5	2.1	2.1	2.0	2.3	2.0
				End-tidal PCO_2 (mmHg)				
1	28.4	28.7	28.1	29.7	29.3	32.2	31.8	32.0
3	29.9		29.8	30.3	29.4	30.0	30.9	30.7
4	38.0	37.5	37.5	39.4	38.0	38.1	37.2	37.0
5	35.4	35.0	33.6	34.6	33.5	33.2	31.7	32.8
6	43.8	43.7	41.3	40.8	40.1	38.2	40.9	40.9
8	41.3	41.5	40.9	40.6	38.0	37.1	39.2	38.6
9	38.3	39.8	39.8	40.4	39.4	36.5	37.6	37.6
10	40.9		40.4	40.3	40.3	39.0	40.6	40.6
Mean	37.0	37.7	36.4	37.0	36.0	35.5	36.2	36.3
SD	5.5	5.4	5.2	4.8	4.6	3.3	4.2	4.0

AMINOPHYLLINE LEG

Subject	Baseline							
	Pre	Post	1	2	3	4	5	Final
				Heart rate (beats/min)				
1	59	61	62	59	60	56	56	60
2	65	62	71	66	68	70	66	63
3	59	60	58	58	61	58	58	59
4	72	77	83	87	82	86	91	97
5	64	79	87	88	79	90	90	97
6	66	74	68	64	61	64	69	71
7	58	55	54	68	57	64	59	60
8	62	63	79	83	79	79	83	74
9	79	87	87	89	87	85	87	87
10	67		66	62	68	64	69	69
Mean	65	69	72	72	70	72	73	74
SD	7	11	12	13	11	12	14	15

Subject	Baseline							
	Pre	Post	1	2	3	4	5	Final
				Systolic blood pressure (mmHg)				
1	106	120	115	124	117	106	117	123
2	128	128	124	128	140	130	128	127
3	94	104	96	103		96	104	
4	106	110	110	111	110	110	116	98
5	137	134	134	130	134	130	140	150
6	82	94	94	86	90	88	90	90
7	112	119	116	110	110	117	115	114
8	120	124	124	126	116	125	119	122
9	108	110	104	110	110	107	108	106
10	112		124	120	126	130		
Mean	111	116	114	115	117	114	115	116
SD	16	13	13	14	15	15	14	19
				Diastolic blood pressure (mmHg)				
1	61	72	73	72	65	63	74	73
2	58	62	72	64	75	75	70	76
3	50	48	54	50	58	53	60	
4	58	64	58	58	63	64	70	66
5	81	74	84	82	78	80	83	90
6	54	58	56	58	58	58	60	66
7	80	69	70	77	72	71	74	70
8	56	55	70	60	64	63	52	56
9	72	78	80	76	74	73	72	71
10	70		66	62	70	66		
Mean	64	64	68	66	68	67	68	71
SD	11	10	10	10	7	8	9	10
				Minute ventilation (l/min)				
1	5.9	8.2	7.8	6.3	6.6	6.2	6.3	7.6
2	9.7	10.7	13.9	10.5	9.3	11.8	10.4	8.8
3	5.0	7.0	6.0	5.6	6.0	4.7	4.7	4.2
4	3.6	5.1	4.1	4.8	4.4	5.1	4.6	4.4
5	5.4	6.3	6.8	7.0	6.3	7.9	8.9	6.5
6	6.3	9.0	6.6	7.4	4.2	6.5	5.1	6.2
7	6.9	8.8	7.7	8.2	7.4	7.7	7.7	7.9
8	4.7	5.7	4.8	4.8	5.0	6.1	5.1	3.8
9	5.0	6.9	6.2	6.4	4.7	5.3	6.2	7.5
10	10.0		12.2	7.7	9.3	9.2	8.3	8.3
Mean	6.3	7.5	7.6	6.9	6.3	7.0	6.7	6.5
SD	2.1	1.8	3.1	1.7	1.9	2.2	2.0	1.8
				End-tidal PCO_2 (mmHg)				
1	39.9	34.8	35.0	35.2	35.9	35.8	34.6	34.3
2	39.5	37.5	37.0	37.5	36.4	36.5	35.9	34.6
3	39.1	35.0	34.6	34.9	34.9	35.8	36.4	35.1
4	36.3	32.1	32.5	33.0	33.2	31.9	32.1	32.2
5	36.2	31.5	31.2	31.9	32.0	31.4	31.2	31.8
6	43.4	34.5	35.7	32.2	38.8	38.0	36.9	37.7
7	37.6	34.3	33.7	32.1	32.6	33.5	33.2	33.5
8	38.2	33.5	34.7	34.2	35.0	35.2	35.2	35.1
9	37.9	31.6	33.1	33.1	33.8	33.1	34.0	31.1
10	40.7		35.3	36.7	36.7	36.4	36.4	36.4
Mean	38.9	33.9	34.3	34.1	34.9	34.8	34.6	34.2
SD	2.2	1.9	1.7	2.0	2.1	2.2	1.9	2.1

CHANGES DURING ADENOSINE INFUSION

PLACEBO LEG

Subject	Control	Adenosine dose (mg/min)				
		2.3	4.3	6.1	8.5	Max (P)
		Peak heart rate (beats/min)				
1	56	60	76	82	86	94
2	75	74	76	79	74	101
3	61	69	69	71	77	87
4	75	73	99	110	116	125
5	80	75	80	88	86	97
6	64	70	76	95	114	114
7	78	83	81	82	100	122
8	66	68	74	89	105	114
9	83	72	101	101	108	108
10	69	68	80	93	93	93
Mean	71	71	81	89	96	106
SD	9	6	11	11	15	13
		Peak systolic blood pressure (mmHg)				
1	112	111	109	116	126	136
2	122	136	128	134	127	152
3	103	102	98	98	106	115
4	108	106	116	122	126	132
5	146	150	156	153	163	171
6	86	98	90	106	102	102
7	120	126	126	130	130	122
8	122	128	136	137	128	130
9	115	108	105	118	110	110
10	120	123	142	140	142	142
Mean	115	119	121	125	126	131
SD	15	17	21	17	18	21
		Trough diastolic blood pressure (mmHg)				
1	52	51	50	50	50	52
2	62	65	66	66	62	59
3	52	48	48	50	44	55
4	54	56	50	53	52	62
5	82	84	83	82	82	82
6	57	54	54	53	56	56
7	66	58	60	60	46	56
8	37	50	42	41	38	30
9	66	60	58	62	66	66
10	62	54	56	48	58	58
Mean	59	58	57	57	55	58
SD	12	10	11	12	13	13
		Peak minute ventilation (mmHg)				
1	6.1	6.8	8.3	12.7	11.8	15.4
2	8.6	9.5	8.9	11.3	11.0	16.6
3	6.0	6.1	6.8	5.9	11.3	16.5
4	4.2	4.3	8.3	10.4	13.9	16.2
5	8.9	6.4	9.7	15.2	12.9	19.6
6	4.3	5.7	5.2	10.9	11.2	11.2
7	6.6	8.0	7.9	10.3	15.1	21.7
8	5.4	6.7	8.7	10.1	17.0	17.3
9	6.0	5.5	8.9	11.8	16.8	16.8
10	9.2	10.4	14.6	22.5	24.9	24.9
Mean	6.6	6.9	8.7	12.1	14.6	17.6
SD	1.8	1.9	2.4	4.3	4.3	3.7

Subject	Control	Adenosine dose (mg/min)				
		2.3	4.3	6.1	8.5	Max (P)
		Trough end-tidal PCO_2 (mmHg)				
1	32.5	28.7	27.3	26.0	26.8	26.2
3	29.1	29.0	28.2	27.7	23.0	19.8
4	38.0	36.5	33.5	31.7	30.4	25.4
5	34.5	33.5	32.3	29.7	26.3	27.2
6	38.0	41.4	36.8	34.2	33.0	33.0
8	37.6	40.3	38.2	34.1	33.0	31.9
9	35.1	40.1	35.2	31.1	30.9	30.9
10	37.8	38.4	34.8	31.6	25.1	25.1
Mean	35.3	36.0	33.3	30.7	28.6	27.4
SD	3.2	5.0	3.9	2.9	3.8	4.4

Subject	Pre	Post	Adenosine dose (mg/min)/time (min)											
			2.3			4.3			Control			Max (P)		
			2.5	5	10	2.5	5	10	2.5	5	10	2.5	5	10
			FEV_1 (l)											
1	3.57	3.35	3.18	3.43	3.32	3.11	3.39	3.30	3.24	3.34	3.28	3.62	3.28	3.23
2	3.52	3.83	3.54	3.42	3.76	3.67	3.53	3.62	3.46	3.17	3.36	2.95	3.13	3.59
3	3.33	3.21	3.16	3.05	3.10	3.19	3.09	2.87	2.95	3.00	3.11	3.10	3.02	3.09
4	4.89	4.87	4.76	4.79	4.85	4.77	4.74	4.80	4.72	4.62	4.61	4.41	4.32	4.42
5	4.67	4.64	4.46	4.65	4.58	4.39	4.49	4.55	4.33	4.19	4.37	4.10	4.23	4.22
6	4.11	3.99	3.84	3.87	3.88	3.90	3.79	3.74	4.06	3.87	3.81	3.92	3.86	3.88
7	2.98	2.83	2.29	2.39	2.37	2.36	2.32	1.87	2.37	2.47	2.14	2.49	2.14	2.42
8	3.06	3.04	2.94	3.07	3.11	2.95	2.96	3.03	3.06	2.98	2.85	2.97	2.76	2.98
9	4.38	4.28		4.13	4.25	4.29	4.38	4.37	4.21	4.24	4.30	4.06	4.17	4.37
10		2.90	2.84	2.74	2.84	2.80	2.76	2.94	2.97	2.83	2.94	2.95	2.95	2.68
Mean	3.83	3.69	3.45	3.55	3.61	3.54	3.55	3.51	3.54	3.47	3.48	3.46	3.39	3.49
SD	0.70	0.74	0.79	0.80	0.80	0.78	0.80	0.90	0.75	0.71	0.78	0.64	0.73	0.72
			FVC (l)											
1	5.07	4.86	4.68	4.78	4.60	4.79	4.87	4.91	4.79	4.84	4.80	5.26	4.89	4.78
2	5.28	5.66	5.72	5.27	5.65	5.55	5.55	5.76	5.34	5.00	5.11	4.64	4.92	5.35
3	3.93	3.87	3.87	3.70	3.73	3.83	3.73	3.50	3.56	3.64	3.78	3.79	3.68	3.77
4	5.98	5.96	5.97	5.98	6.03	5.95	5.95	6.00	6.00	5.81	5.82	6.00	5.80	5.90
5	5.78	5.76	5.58	5.70	5.65	5.53	5.57	5.64	5.34	5.38	5.49	5.25	5.37	5.38
6	4.91	4.73	4.69	4.72	4.84	4.84	4.82	4.85	4.95	4.73	4.60	4.74	4.72	4.60
7	3.50	3.47	3.30	3.42	3.46	3.40	3.40	3.41	3.47	3.36	3.37	3.19	3.13	3.28
8	3.73	3.74	3.28	3.52	3.91	3.49	3.66	3.76	3.83	3.75	3.54	3.69	3.49	3.69
9	5.44	5.48		5.23	5.38	5.48	5.61	5.55	5.44	5.44	5.48	5.30	5.35	5.49
10		3.87	3.85	3.77	3.81	3.75	3.68	3.91	3.93	3.80	4.02	3.87	3.93	3.55
Mean	4.85	4.74	4.55	4.61	4.71	4.66	4.68	4.73	4.67	4.58	4.60	4.57	4.53	4.58
SD	0.91	0.95	1.04	0.95	0.94	0.97	0.98	1.00	0.90	0.87	0.88	0.90	0.91	0.95
			FEV_1/FVC (%)											
1	70	69	68	72	72	65	70	67	68	69	68	69	67	68
2	67	68	62	65	67	66	64	63	65	63	66	64	64	67
3	85	83	82	82	83	83	83	82	83	82	82	82	82	82
4	82	82	80	80	80	80	80	80	79	80	79	74	74	75
5	81	81	80	82	81	79	81	81	81	78	80	78	79	78
6	84	84	82	82	80	81	79	77	82	82	83	83	82	84
7	85	82	69	70	68	69	68	55	68	74	64	78	68	74
8	82	81	90	87	80	85	81	81	80	79	81	80	79	81
9	81	78		79	79	78	78	79	77	78	78	77	78	80
10		75	74	73	75	75	75	75	76	74	73	76	75	75
Mean	80	78	76	77	77	76	76	74	76	76	75	76	75	76
SD	7	6	9	7	6	7	6	9	7	6	7	6	6	6

Subject	Pre	Post	Adenosine dose (mg/min)/time (min)											
			2.3			4.3			Control			Max (P)		
			2.5	5	10	2.5	5	10	2.5	5	10	2.5	5	10
			$FEF_{25-75\%}$ (l/s)											
1	2.49	2.41	2.22	2.51	2.60	1.96	2.38	2.21	2.30	2.26	2.25	2.49	2.15	2.06
2	5.25	4.37	2.22	2.20	2.50	2.50	2.24	2.25	2.20	2.01	2.21	1.88	1.97	2.44
3	4.02	4.03	3.69	3.13	3.27	3.80	3.30	2.89	3.25	3.21	3.68	3.70	3.32	3.27
4	4.32	4.34	4.16	4.18	4.23	4.13	4.12	4.20	4.07	4.09	4.05	3.30	3.31	3.70
5	4.20	4.17	4.01	4.21	4.09	3.95	4.05	4.12	4.02	3.69	3.92	3.69	3.82	3.74
6	4.79	4.33	4.33	4.15	4.08	3.98	4.00	3.85	4.10	3.97	4.04	4.17	4.12	4.26
7	3.24	2.76	1.62	1.75	1.74	1.72	1.73	1.14	1.73	1.93	1.48	2.14	1.33	1.99
8	3.13	3.27	3.90	3.80	2.87	3.21	2.91	2.88	2.80	2.81	2.71	2.75	2.44	2.79
9	4.16	4.01		3.88	3.96	4.03	3.97	4.05	3.96	4.01	4.00	3.76	3.92	4.12
10		2.33	1.99	1.81	2.05	2.04	2.00	2.21	2.25	1.99	1.98	2.31	2.15	2.04
Mean	3.96	3.60	3.13	3.16	3.14	3.13	3.07	2.98	3.07	3.00	3.03	3.02	2.85	3.04
SD	0.86	0.83	1.08	1.01	0.92	0.98	0.94	1.04	0.92	0.91	1.01	0.80	0.96	0.89

Subject	Pre	Post	Base-line 1	Control	Adenosine dose (mg/min)				
					2.3	4.3	6.1	8.5	Max (P)
					Venous plasma adenosine concentration (μM)				
1	0.10	0.09	0.06	0.14	0.04	0.10	0.09		0.09
2	0.10	0.10	0.13	0.08	0.08	0.07	0.08	0.07	0.20
3	0.04	0.03	0.03	0.10	0.08	0.09	0.07	0.05	0.04
7	0.03	0.05	0.05	0.04	0.09	0.03	0.04	0.04	0.07
10	0.14	0.14	0.07	0.07	0.09	0.07	0.06	0.09	0.09
*	0.02	0.02	0.04	0.03	0.03	0.04	0.08	0.04	0.04
Mean	0.07	0.07	0.06	0.07	0.07	0.07	0.07	0.06	0.09
SD	0.05	0.04	0.04	0.04	0.03	0.03	0.02	0.02	0.07

*, patient with asthma.

TIMECOURSE OF CHANGES (Maximum dose of adenosine)

Subject	Time (min)							
	0	1	2	3	4	5	6	7
				Minute ventilation (l/min)				
1	5.0	15.4	8.1	6.1	9.3	6.4	3.5	3.4
2	8.7	12.2	12.0	16.6	12.8	13.5	7.6	4.4
4	3.5	13.3	10.7	16.2	12.2	9.4	3.9	2.7
5	7.0	19.6	15.4	12.6	11.3	9.4	5.6	4.5
6	3.7	6.8	11.2	9.3	7.3	5.1	6.5	4.7
7	6.4	21.7	21.1	15.4	11.7	13.5	3.4	4.3
8	5.2	14.8	17.3	11.9	8.8	8.8	2.9	1.3
10	9.0	18.3	24.9	24.3	16.3	22.0	10.3	7.7
Mean	6.1	15.3	15.1	14.0	11.2	11.0	5.5	4.1
SD	2.1	4.7	5.7	5.5	2.8	5.4	2.6	1.8
				End-tidal PCO_2 (mmHg)				
1	32.0	27.8	26.2	27.3	26.3	27.7	31.7	32.0
4	37.0	30.7	28.1	25.8	25.9	25.4	28.1	30.2
5	32.8	29.2	29.7	28.1	27.2		29.3	30.0
6	40.9	38.1	34.8	34.4	33.0	33.1	34.3	33.4
8	38.6	37.0	32.8	31.9	32.6	32.2	34.4	36.3
10	40.6	36.7	27.9	26.1	27.4	25.1	29.2	32.8
Mean	37.0	33.3	29.9	28.9	28.7	28.7	31.2	32.5
SD	3.8	4.5	3.3	3.5	3.2	3.8	2.7	2.3

CHANGE IN FRC (l)

Subject	Time (min)						
	1	2	3	4	5	6	7
			Maximum dose of adenosine				
1	-0.04	0.12	0.21	0.18	0.18	0.12	0.07
2	-0.08	-0.03	0.20	-0.03	0.00	0.17	0.03
4	0.08	0.05	-0.11	0.02	-0.08	-0.20	-0.17
5	0.92	0.96	0.48	0.52	0.48	-0.20	-0.48
6	0.28	0.35	0.22	0.30	0.00	-0.10	-0.25
7	0.23	0.36	0.33	0.17	0.13	0.00	0.10
8	0.09	0.09	0.00	-0.03	0.00	-0.06	-0.12
10	0.00	0.00	0.00	-0.09	-0.16	-0.19	-0.06
Mean	0.19	0.24	0.17	0.13	0.07	-0.06	-0.11
SD	0.32	0.33	0.19	0.21	0.20	0.14	0.19
			Control infusion.				
1	-0.05	-0.14	-0.25	-0.28	-0.37	-0.35	-0.33
2	0.03	0.03	0.00	-0.03	-0.01	-0.03	-0.03
4	-0.01	0.01	0.01	-0.01	0.01	-0.01	-0.05
5	-0.04	0.02	0.04	0.04	0.04	0.04	-0.10
6	-0.02	0.02	-0.06	0.05	-0.02	0.06	-0.03
7	0.07	-0.03	-0.07	0.07	0.12	0.27	0.30
8	0.03	-0.01	-0.01	-0.03	-0.06	-0.06	-0.03
10	-0.13	-0.13	-0.13	-0.09	-0.13	-0.13	-0.09
Mean	-0.02	-0.03	-0.06	-0.04	-0.05	-0.03	-0.05
SD	0.06	0.07	0.09	0.11	0.15	0.17	0.17

AMINOPHYLLINE LEG

Subject	Control	Adenosine dose (mg/min)				
		2.3	4.3	6.1	8.5	Max (P)
			Peak heart rate (beats/min)			
1	60		64	64	73	85
2	68	68	71	98	87	98
3	62	62	61	60	62	75
4	92	84	96	114	156	161
5	90	88	91	89	98	99
6	72	72	71	75	93	93
7	63	64	69	71	82	109
8	84	82	82	83	92	134
9	90	92	98	102	107	107
10	69	64	67	72	90	90
Mean	75	75	77	83	94	105
SD	13	11	14	18	25	25
			Peak systolic blood pressure (mmHg)			
1	122		118	118	119	117
2	132	126	142	136	130	142
3	104	105	103	102	100	110
4	113	116	116	116	130	125
5	144	144	142	140	144	138
6	88	90	86	94	94	94
7	118	122	117	124	122	130
8	126	130	126	130	130	136
9	108	111	109	114	112	112
10	126	130	128	122	130	130
Mean:	118	119	119	120	121	123
SD:	16	16	17	14	15	15

Subject	Control	Adenosine dose (mg/min)				
		2.3	4.3	6.1	8.5	Max (P)
		Trough diastolic blood pressure (mmHg)				
1	62		68	61	57	55
2	72	64	64	62	68	60
3	49	51	51	50	46	45
4	52	60	55	54	60	62
5	70	83	80	72	70	72
6	55	44	56	50	40	40
7	68	72	74	69	67	54
8	48	57	54	52	54	43
9	72	74	78	70	70	70
10	54	58	64	58	64	64
Mean	60	63	64	60	60	57
SD	10	12	10	8	10	11
		Peak minute ventilation (l/min)				
1	7.6		8.2	7.5	10.2	10.9
2	10.8	11.9	12.8	12.0	14.2	17.0
3	5.7	6.5	6.8	6.4	7.2	7.8
4	5.3	4.7	7.1	7.2	14.0	12.9
5	7.4	7.6	7.7	9.9	11.4	12.1
6	7.2	8.6	8.1	7.1	8.8	8.8
7	8.6	9.9	9.0	10.8	11.1	18.0
8	3.8	5.2	4.7	6.6	7.1	9.9
9	7.5	7.4	5.3	9.6	8.7	8.7
10	10.1	12.1	9.8	11.7	20.6	20.6
Mean	7.4	8.2	7.9	8.9	11.3	12.7
SD	2.1	2.7	2.3	2.2	4.1	4.4
		Trough end-tidal PCO_2 (mmHg)				
1	34.3		34.0	35.0	32.5	30.8
2	36.1	37.1	36.6	35.6	34.3	30.8
3	35.9	34.1	34.7	32.8	32.8	31.2
4	31.1	31.1	30.5	31.0	28.2	27.6
5	30.7	31.7	30.8	29.2	29.5	28.3
6	37.1	34.3	31.6	37.5	31.9	31.9
7	32.3	33.7	30.9	32.3	31.5	27.3
8	34.8	34.2	34.4	34.5	32.6	30.8
9	31.2	29.6	31.8	29.2	29.3	29.3
10	35.3	36.0	35.2	33.6	29.5	29.5
Mean	33.9	33.5	33.1	33.1	31.2	29.7
SD	2.4	2.4	2.2	2.7	2.0	1.6

Subject	Pre	Post	Adenosine dose (mg/min)/time (min)											
			2.3			4.3			Control			Max (A)		
			2.5	5	10	2.5	5	10	2.5	5	10	2.5	5	10
								FEV_1 (l)						
1	3.11	3.43	3.37	3.29	3.50	3.50	3.42	3.42	3.12	3.00	3.26	3.25	3.44	3.25
2	3.54	4.04	3.71	4.00	4.20	4.00	3.83	4.07	3.60	3.60	3.48	3.95	3.70	3.86
3	3.21	3.30				3.24	3.26	3.31	3.21	3.30	3.38	3.18	3.12	3.01
4	4.60	4.80	4.77	4.79	4.77	4.71	4.60	4.89	4.93	4.96	4.89	5.03	4.96	5.09
5	4.30	4.51	4.57	4.45	4.69	4.60	4.49	4.50	4.45	4.53	4.49	4.49	4.38	4.29
6	4.04	4.20	4.02	4.14	4.19	4.17	4.19	4.22	4.21	4.18	4.06	3.85	3.81	4.13
7	2.89	2.90	3.03	2.59	2.92	2.98	2.97	2.89	2.94	3.07	3.07	2.89	2.80	2.81
8	3.15	3.18	3.09	3.09	3.21	3.22	3.18	3.20	3.21	3.20	3.16	3.07	3.06	2.88
9	4.16	4.24	4.32	4.33	4.19	4.29	3.99	4.28	4.33	4.07	3.82	4.08	4.22	4.21
10	2.86	3.05	3.01	2.84	2.98	3.03	3.08	3.08	3.18	3.23	3.17	3.17	3.18	3.12
Mean	3.59	3.77	3.77	3.72	3.85	3.77	3.70	3.79	3.72	3.71	3.68	3.70	3.67	3.67
SD	0.64	0.67	0.69	0.79	0.71	0.66	0.60	0.69	0.70	0.68	0.62	0.70	0.68	0.76

Subject	Pre	Post	Adenosine dose (mg/min)/time (min)											
			2.3			4.3			Control			Max (A)		
			2.5	5	10	2.5	5	10	2.5	5	10	2.5	5	10
						FVC (l)								
1	4.64	5.02	4.95	4.88	5.11	5.14	4.90	4.90	4.72	4.79	4.97	4.87	4.81	4.93
2	5.25	5.72	5.38	5.80	5.82	5.87	5.73	6.04	5.09	5.47	5.15	5.93	5.58	5.58
3	3.86	3.95				3.91	3.92	3.91	3.78	3.92	3.98	3.77	3.71	3.64
4	5.74	5.85	5.91	5.84	5.87	5.80	5.62	5.88	5.81	5.95	5.83	6.04	6.05	6.10
5	5.52	5.69	5.68	5.53	5.90	5.74	5.64	5.67	5.62	5.69	5.68	5.59	5.73	5.56
6	4.99	5.01	4.84	4.89	5.09	4.95	4.82	4.92	4.85	4.81	4.72	4.40	4.65	4.96
7	3.55	3.51	3.57	3.36	3.56	3.48	3.48	3.50	3.35	3.46	3.49	3.43	3.12	3.33
8	3.62	3.87	3.73	3.72	3.95	3.84	3.72	3.77	3.56	3.95	3.91	3.47	3.66	3.21
9	5.26	5.19	5.32	5.32	5.19	5.32	4.96	5.23	5.33	5.00	4.74	5.12	5.17	5.19
10	3.86	4.05	4.00	3.80	3.87	3.99	3.97	3.93	4.25	4.28	4.24	4.20	4.14	4.17
Mean	4.63	4.79	4.82	4.79	4.93	4.80	4.68	4.78	4.64	4.73	4.67	4.68	4.66	4.67
SD	0.84	0.87	0.86	0.94	0.92	0.92	0.85	0.94	0.87	0.82	0.77	0.98	0.99	1.02
						FEV_1/FVC (%)								
1	67	68	68	67	68	68	70	70	66	63	66	67	72	66
2	67	71	69	69	72	68	67	67	71	66	68	67	66	69
3	83	84				83	83	85	85	84	85	84	84	83
4	80	82	81	82	81	81	82	83	85	83	84	83	82	83
5	78	79	80	80	79	80	80	79	79	80	79	80	76	77
6	81	84	83	85	82	84	87	86	87	87	86	88	82	83
7	81	83	85	77	82	86	85	83	88	89	88	84	90	84
8	87	82	83	83	81	84	85	85	90	81	81	88	84	90
9	79	82	81	81	81	81	80	82	81	81	81	80	82	81
10	74	75	75	75	77	76	78	78	75	75	75	75	77	75
Mean	78	79	78	78	78	79	80	80	81	79	79	80	79	79
SD	7	6	6	6	5	6	7	6	8	9	8	8	7	7
						$FEF_{25-75\%}$ (l/s)								
1	2.32	2.40	2.33	2.27	2.49	2.44	2.44		2.13	1.85	2.11	2.02	2.79	2.13
2	2.35	2.98	2.54	2.72	3.17	2.80	2.51	2.77	2.57	2.34	2.26	2.64	2.47	2.77
3	3.86	4.00				3.97	4.04	4.08	3.89	4.02	4.09	3.88	3.85	3.19
4	4.25	4.32	4.22	4.28	4.25	4.17	4.16	4.41	4.54	4.50	4.42	4.54	4.40	4.58
5	3.87	4.04	4.18	4.05	4.22	4.08	4.03	4.07	3.96	4.05	3.97	4.02	3.75	3.70
6	4.37	4.51	4.21	4.36	4.37	4.44	4.61	4.50	4.51	4.51	4.56	4.40	4.17	4.30
7	2.98	3.09	3.27	3.86	3.10	3.25	3.69	2.92	3.93	3.88	3.20	3.98	4.05	2.72
8	3.94	4.23		3.32	3.20	3.77	3.86	3.88	4.25	3.20	3.15	4.05	3.23	3.94
9	4.17	4.28	4.21	4.15	4.06	4.11	3.90	4.17	4.22	4.05	3.95	3.93	4.15	4.07
10	2.14	2.33	2.24	2.04	2.41	2.30	2.54	2.63	2.30	2.43	2.30	2.37	2.47	2.26
Mean	3.42	3.62	3.40	3.45	3.47	3.53	3.58	3.71	3.63	3.48	3.40	3.58	3.53	3.37
SD	0.88	0.83	0.91	0.90	0.77	0.78	0.78	0.73	0.93	0.96	0.93	0.89	0.73	0.87

Subject	Pre	Post	Base-line 1	Control	Adenosine dose (mg/min)				
					2.3	4.3	6.1	8.5	Max (A)
				Venous plasma adenosine concentration (μM)					
1	0.03	0.05	0.04	0.06		0.04	0.04	0.04	0.08
2	0.09		0.12		0.12				0.15
3	0.06	0.05	0.04	0.08	0.07	0.09	0.05	0.05	0.09
7	0.05	0.09	0.08	0.06	0.09	0.06	0.08	0.11	0.08
10	0.09	0.11	0.09	0.09	0.10	0.10	0.30	0.11	0.11
*	0.09	0.06		0.08	0.07	0.06	0.07	0.09	0.09
Mean	0.07	0.07	0.07	0.07	0.09	0.07	0.11	0.08	0.10
SD	0.03	0.03	0.03	0.01	0.02	0.03	0.11	0.03	0.03

*, patient with asthma.

APPENDIX 4 - MAIN INDIVIDUAL DATA FOR CHAPTER 6

Patient details are shown in Chapter 6.

Subject	Baseline	Adenosine dose (mg/min)						2' Post
		4.3	6.1	8.5	11.9	16.8	Max	
				Plasma adenosine concentration (μM)				
1	0.12	0.12	0.14	0.14	0.69	1.40	1.40	
2	0.10	0.06	0.06	0.73	2.04		2.04	0.10
3	0.09	0.09	0.14	1.12			1.12	0.03
4	0.05	0.28	1.24				1.24	0.06
5	0.04	0.04	0.04	0.06	0.42		0.42	0.03
6	0.07	0.08	0.36	1.48			1.48	0.09
7	0.05	0.02	0.70				0.70	0.09
Mean	0.07	0.10	0.38				1.20	0.07
SD	0.03	0.08	0.44				0.53	0.03
				Respiratory rate (breaths/min)				
1	16	15	16	17	17	17	17	17
2	18	19	14	17	17		17	9
3	17	13	12	20			20	21
4	14	17	18				18	9
5	19	9	11	19	12		12	10
6	14	14	14	14			14	5
7	23	23	25				25	13
Mean	17	16	16				18	12
SD	3	4	5				4	5
				Tidal volume (l)				
1	0.40	0.48	0.53	0.64	0.69	0.71	0.71	0.40
2	0.46	0.45	0.85	0.89	0.80		0.80	0.72
3	0.24	0.44	0.54	0.29			0.29	0.17
4	0.22	0.42	0.84				0.84	0.36
5	0.38	0.56	0.59	0.59	0.85		0.85	0.69
6	0.42	0.41	0.67	0.80			0.80	0.91
7	0.16	0.16	0.31				0.31	0.28
Mean	0.33	0.42	0.62				0.66	0.50
SD	0.12	0.12	0.19				0.25	0.27
				Minute ventilation (l/min)				
1	6.4	7.2	8.7	10.8	11.9	11.8	11.8	6.7
2	8.1	8.8	11.8	15.0	13.8		13.8	6.5
3	4.1	5.5	6.4	5.9			5.9	3.5
4	3.2	7.2	15.2				15.2	3.3
5	7.3	5.2	6.6	11.4	10.5		10.5	6.7
6	5.8	5.8	9.5	11.3			11.3	4.6
7	3.7	3.7	7.7				7.7	3.8
Mean	5.5	6.2	9.4				10.9	5.0
SD	1.9	1.7	3.2				3.3	1.6
				Heart rate (beats/min)				
1	62	62	61	62	70	75	75	74
2	56	56	56	58	70		70	64
3	70	64	69	80			80	65
4	44	45	58				58	49
5	82	86	86	80	95		95	96
6	54	53	54	70			70	56
7	60	53	55				55	65
Mean	61	60	63				72	67
SD	12	13	11				14	15

Subject	Baseline	Adenosine dose (mg/min)						2' Post
		4.3	6.1	8.5	11.9	16.8	Max	
				Systolic blood pressure (mmHg)				
1	133	136	137	137	139	145	145	147
2	114	109	100	108	94		94	104
3	156	145	149	148			148	151
4	132	120	120				120	123
5	158	157	160	147	154		154	165
6	160	153	154	143			143	144
7	115	139	140				140	137
Mean	138	137	137				135	139
SD	20	17	21				21	20
				Diastolic blood pressure (mmHg)				
1	76	77	75	73	77	79	79	80
2	61	58	55	56	50		50	56
3	77	70	73	74			74	69
4	64	58	57				57	60
5	107	108	111	99	109		109	115
6	71	66	62	65			65	61
7	55	64	61				61	65
Mean	73	72	71				71	72
SD	17	17	19				20	20
				Mean blood pressure (mmHg)				
1	94	98	97	100	102	107	107	109
2	78	79	79	80	70		70	81
3	102	103	102	102			102	106
4	86	86	78				78	89
5	124	129	132	118	135		135	142
6	102	92	94	98			98	98
7	85	92	94				94	103
Mean	96	97	97				98	104
SD	15	16	18				21	19

APPENDIX 5 - MAIN INDIVIDUAL DATA FOR CHAPTER 7

Patient details are shown in Chapter 7.

Subject	Baseline	Control	Adenosine dose (mg/min)			5 min post
			4.3	6.1	Maximum	
			Heart rate (beats/min)			
1	56	58	56	62	73	68
2	58	56	55	84	84	59
3	55	55	59	70	81	61
4	49	47	50	55	61	53
5	58	56	58	75	98	66
6	100	101	122	136	136	104
7	68	70	63	81	89	70
8	87	85	93	106	106	88
9	72	67	65	79	92	75
10	72	64	66	94	95	70
11	82	80	105	117	117	82
12	62	60	70	75	85	72
13	57	59	62	78	78	63
14	124	111	120	145	145	95
15	56	56	56	58	75	56
16	47	45	43	51	62	52
Mean	69	67	68	85	92	71
SD	21	19	30	28	24	15
			Mean systemic blood pressure (mmHg)			
1	104	104	104	106	96	92
2	82	90	90	88	88	86
3	96	94	100	92	92	88
4	84	100	85	80	84	84
5	100	96	104	112	104	104
8	114	117	96	104	104	108
9	70	95	100	107	71	96
10	102	87	109	101	89	104
11	99	94	91	93	93	93
12	81	83	88	80	83	92
13	88	88	91	94	94	94
14	109	107	100	90	90	103
15	86	91	98	93	84	91
16	100	107	108	119	118	110
Mean	94	97	97	97	92	96
SD	12	9	8	12	11	8
			Cardiac index (l/min/m²)			
1	3.7	3.6	3.6	4.9	6.0	5.6
2	4.3	4.2	5.0	6.8	6.8	5.0
3	4.0	3.9	3.8	5.7	6.0	4.6
4	3.9	3.8	3.9	4.0	5.5	4.8
5	4.7	4.6	4.9	6.7	10.0	6.2
6	4.0	3.8	4.6	5.6	5.6	3.9
7	2.7	2.7	2.8	3.8	4.1	2.8
8	2.9	3.1	3.7	4.4	4.4	3.5
9	2.4	2.3	2.2	3.0	3.6	2.9
10	2.6	2.4	2.5	3.9	4.4	2.7
11	3.8	3.4	4.9	5.2	5.2	3.6
12	2.8	3.0	3.0	3.7	4.3	3.5
13	2.7	2.6	2.3	3.0	3.0	2.6
14	4.8	5.0	5.3	5.3	5.3	4.6
15	2.6	2.7	2.5	3.0	4.4	2.9
16	2.5	2.3	2.3	2.9	3.7	2.8
Mean	3.4	3.3	3.6	4.5	5.1	3.9
SD	0.8	0.8	1.1	1.3	1.7	1.1

Subject	Baseline	Control	Adenosine dose (mg/min)			5 min post
			4.3	6.1	Maximum	
			Mean right atrial pressure (mmHg)			
1	3	4	4	5	5	5
2	-2	-1	-1	0	0	-1
3	3	3	2	1	3	3
4	1	2	1	1	4	1
5	-2	-1	-1	-3	-2	-1
6	2	2	2	4	4	2
7	0	3	1	1	1	2
8	4	2	5	3	3	3
9	4	4	3	4	4	4
10	3	4	4	4	4	3
11	5	5	6	6	6	5
12	-2	-1	-2	-2	6	1
13	3	3	3	2	2	4
14	-1	-2	0	-3	-3	0
15	-4	-3	-3	-3	-3	-3
16	0	2	1	1	0	2
Mean	1	2	2	1	2	2
SD	3	2	3	3	3	2
			Mean pulmonary artery pressure (mmHg)			
1	12	16	15	23	20	16
2	12	11	8	18	18	10
3	10	15	12	13	19	15
4	10	14	8	8	8	7
5	9	9	8	10	16	11
6	12	11	15	23	23	11
7	7	8	6	7	13	10
8	7	8	10	19	19	13
9	8	11	11	12	10	17
10	14	13	14	12	14	14
11	14	14	16	20	20	18
12	12	15	10	11	27	22
13	13	13	13	15	15	15
14	7	7	8	9	9	12
15	8	8	8	6	11	8
16	10	12	13	13	19	17
Mean	10	12	11	14	16	14
SD	2	3	3	5	5	4
			Mean pulmonary capillary wedge pressure & (LVEDP) (mmHg)			
1	8	10	10	13	16	10
2	2	1	0	8	8	2
3	4	7	5	4	14	8
4	2	3	2	3	5	2
5	1	2	3	3	5	2
6	6	6	13	15	15	5
7	1	3	2	2	7	6
8	2	1	4	13	13	5
9	1 (0)	6	7	6	5 (11)	10
10	7 (10)	5	6	2	7 (8)	5
11	4 (7)	8	10	11	11 (9)	9
12	2 (4)	4	2	4	20 (26)	8
13	5 (10)	5	6	11	11 (27)	10
14	4 (-6)	3	2	3	3 (-1)	5
15	-1 (2)	0	2	0	2 (10)	0
16	4 (12)	6	9	10	12 (19)	12
Mean	3 (5)	4	5	7	10 (14)	6
SD	2 (6)	3	4	5	5 (10)	4

Subject	Baseline	Control	Adenosine dose (mg/min)			5 min post
			4.3	6.1	Maximum	
Systemic vascular resistance (dyne.s.cm^{-5})						
1	1111	1108	1134	839	608	624
2	862	941	802	564	564	758
3	949	949	1044	642	609	755
4	836	1015	854	775	578	689
5	931	903	934	739	457	734
8	1645	1637	1052	1000	1000	1304
9	1066	1501	1710	1294	710	1189
10	1714	1782	1896	1101	852	1652
11	1131	1211	806	778	778	1141
12	1074	1002	1102	796	642	930
13	1388	1429	1649	1341	1341	1506
14	1186	1124	969	891	891	1151
15	1366	1360	1597	1249	774	1279
16	1660	1822	1894	1668	1306	1588
Mean	1209	1270	1247	977	794	1093
SD	299	319	408	314	267	350
Pulmonary vascular resistance (dyne.s.cm^{-5})						
1	44	66	57	83	27	43
2	103	103	70	64	64	70
3	61	83	75	64	34	62
4	81	114	61	49	22	42
5	73	65	44	45	47	63
6	72	63	21	68	68	73
7	101	84	64	60	67	66
8	75	100	71	59	59	99
9	113	82	70	75	53	90
10	121	148	144	113	70	147
11	120	68	57	81	81	117
12	129	131	98	68	58	143
13	131	134	131	58	58	84
14	32	41	58	58	58	78
15	137	116	95	78	80	109
16	100	104	71	42	77	74
Mean	93	94	74	67	58	85
SD	32	30	31	17	18	31
PR interval (ms)						
1	162	164			166	
2	158	164			164	
3	192	190			189	
4	223	233			234	
5	203	198			193	
7	194	199			198	
8	171	176			178	
9	185	168			191	
10	154	158			174	
11	194	190			159	
12	189	182			185	
13	169	178			179	
15	175	181			187	
16	204	200			212	
Mean	184	184			186	
SD	20	20			20	

Subject	Baseline	Control	Adenosine dose (mg/min)			5 min post
			4.3	6.1	Maximum	
			Minute ventilation (l/min)			
2	2.3	2.2	6.2	6.0	6.0	3.5
3	3.4	3.5	4.1	5.9	7.5	1.9
4	7.4	8.4	4.7	10.0	11.8	3.9
5	3.0	3.0	4.0	5.9	8.2	3.2
6	3.3	2.4	7.2	6.8	6.8	1.9
7	4.3	4.8	9.4	9.4	9.7	3.1
8	3.7	3.7	7.1	6.2	6.2	3.4
9	7.2	7.3	8.3	11.4	15.3	5.0
10	6.7	4.8	5.2	11.3	6.6	3.8
11	4.1	4.9	11.8	10.1	10.1	3.4
12	8.6	7.7	9.8	9.5	11.3	5.9
Mean	4.9	4.8	7.1	8.4	9.0	3.5
SD	2.1	2.1	2.5	2.3	2.9	1.2
			Oxygen consumption (ml/kg/m²)			
1	4.6	4.3	4.1	5.1	4.9	6.8
2	4.2	4.3	4.5	4.9	4.9	5.7
3	4.8	4.7	4.3	4.7	4.6	6.1
4	5.5	5.3	5.8	5.2	5.4	5.9
5	5.6	5.5	5.5	5.5	6.2	7.0
6	3.2	3.4	3.1	4.0	4.0	3.7
7	2.6	2.8	2.5	2.9	3.2	2.9
8	2.6	2.7	2.5	2.8	2.8	3.3
9	2.6	2.5	2.4	2.6	2.4	2.7
10	2.8	3.0	2.8	3.3	4.2	3.6
11	3.0	2.9	2.6	2.5	2.5	2.5
12	3.0	3.3	3.3	2.9	3.0	3.3
13	3.2	3.0	2.8	3.1	3.0	3.3
14	2.8	3.0	2.9	3.0	3.0	3.5
15	2.2	2.5	2.1	2.3	2.3	2.4
16	2.5	2.5	2.5	2.8	2.7	2.4
Mean	3.4	3.5	3.4	3.6	3.7	4.0
SD	1.1	1.0	1.1	1.1	1.2	1.6

Subject	B	Max	5' post	B	Max	5' post	B	Max	5' post
	PO_2 (mmHg)			PCO_2 (mmHg)			pH		
9	66	77	71	40	34	42	7.41	7.47	7.39
10	99	67	86	35	29	31	7.44	7.49	7.47
11	80	83	63	39	28	34	7.41	7.50	7.44
12	72	83	73	47	34	41	7.36	7.45	7.39
13	73	77	73	36	32	39	7.39	7.44	7.39
14	103	102	90	31	26	30	7.42	7.46	7.42
15	83	94	68	43	34	42	7.36	7.44	7.37
16	86	97	66	42	28	38	7.34	7.45	7.36
Mean	83	85	74	39	31	37	7.39	7.46	7.40
SD	13	12	9	5	3	5	0.03	0.02	0.04

B, baseline; Max, maximum dose of adenosine; 5' Post, 5 min following the infusion.

APPENDIX 6 - MAIN INDIVIDUAL DATA FOR CHAPTER 8

Patient details are shown in Chapter 8.

BASELINE VALUES

Subject	Placebo	Adenosine dose (mg/min)						
		2.3	4.3	6.1	8.5	11.9	16.8	Maximum
				Heart rate (beats/min)				
1	82	78	80	71	85	81		81
2	100	91	90					90
3	87	82	91	84				84
4	80	79	78	80				80
5	83	76	78	87	94			94
6	91	85	85	79		86	80	80
Mean	87	82	84					85
SD	7	5	6					6
				Systolic blood pressure (mmHg)				
1	117	118	124	118	129	131		131
2	153	156	143					143
3	157	167	148	142				142
4	135	137	135	140				140
5	105	114	108	110	107			107
6	130	139	137	134		135	132	132
Mean	133	139	133					133
SD	20	21	14					13
				Diastolic blood pressure (mmHg)				
1	58	59	62	53	55	61		61
2	82	78	76					76
3	80	85	76	76				76
4	64	64	65	67				67
5	48	53	51	51	50			50
6	58	59	61	59		60	57	57
Mean	65	66	65					65
SD	13	12	10					10
				Minute ventilation (l/min)				
1	6.9	7.3	7.3	7.4	6.9	7.9		7.9
2	4.5	4.7	4.3					4.3
3	8.2	8.4	7.7	8.1				8.1
4	6.6	6.6	6.7	6.8				6.8
5	5.8	6.1	6.2	6.7	6.5			6.5
6	12.8	10.8	9.2	11.4		11.1	13.6	13.6
Mean	7.5	7.3	6.9					7.9
SD	2.9	2.1	1.6					3.1
				PaO_2				
1	58	61	60	58	58	59		59
2	65	61	65					65
3	59	64	63	63				63
4				62				62
5	50	48	52	45	49			49
6	62	64	65	65		62	59	59
Mean	59	60	61					60
SD	6	7	5					6

Subject	Placebo	Adenosine dose (mg/min)						
		2.3	4.3	6.1	8.5	11.9	16.8	Maximum
				$PaCO_2$				
1	52	51	53	51	53	51		51
2	45	45	44					44
3	41	40	41	41				41
4				49				49
5	59	58	57	60	61			61
6	34	36	34	34		35	34	34
Mean	46	46	46					47
SD	10	9	9					9
				pH				
1	7.37	7.37	7.36	7.38	7.38	7.37		7.37
2	7.36	7.37	7.36					7.36
3	7.38	7.39	7.38	7.39				7.39
4				7.35				7.35
5	7.35	7.36	7.36	7.35	7.35			7.35
6	7.45	7.45	7.45	7.46		7.46	7.46	7.46
Mean	7.38	7.39	7.38					7.38
SD	0.04	0.03	0.04					0.04

VALUES DURING ADENOSINE INFUSION

Subject	Placebo	Adenosine dose (mg/min)						
		2.3	4.3	6.1	8.5	11.9	16.8	Maximum
				Heart rate (l/min)				
1	72	85	78	75	71	72		72
2	93	93	100					100
3	82	84	92	122				122
4	77	80	82	81				81
5	79	78	80	88	99			99
6	83	83	83	81		81	69	69
Mean	81	84	86					90
SD	7	5	8					20
				Systolic blood pressure (mmHg)				
1	116	123	125	121	126	147		147
2	149	158	162					162
3	149	154	162	206				206
4	137	141	139	150				150
5	102	113	112	116	124			124
6	128	140	138	135		133	149	149
Mean	130	138	140					156
SD	19	18	20					27
				Diastolic blood pressure (mmHg)				
1	56	58	57	59	61	63		63
2	80	80	87					87
3	72	81	113	114				114
4	66	67	69	72				72
5	48	52	51	52	53			53
6	54	61	59	56		57	59	59
Mean	62	66	72					74
SD	12	12	23					23

Subject	Placebo	Adenosine dose (mg/min)						
		2.3	4.3	6.1	8.5	11.9	16.8	Maximum
				Respiratory rate (breaths/min)				
1	32	27	30	25	27	33		33
2	17	18	19					19
3	23	22	25	32				32
4	20	22	22	20				20
5	17	20	17	19	18			18
6	23	24	23	24		23	19	19
Mean	22	22	23					23
SD	6	3	5					7
				Tidal volume (l)				
1	0.26	0.35	0.27	0.45	0.38	0.38		0.38
2	0.29	0.27	0.29					0.29
3	0.36	0.40	0.39	0.28				0.28
4	0.33	0.34	0.36	0.43				0.43
5	0.40	0.40	0.45	0.44	0.52			0.52
6	0.55	0.45	0.44	0.49		0.53	0.67	0.67
Mean	0.36	0.37	0.37					0.43
SD	0.10	0.06	0.07					0.15
				Minute ventilation (l/min)				
1	8.4	9.4	8.1	11.5	10.2	12.2		12.2
2	4.9	4.7	5.5					5.5
3	8.6	8.8	9.9	9.0				9.0
4	6.6	7.4	8.1	8.7				8.7
5	6.8	8.3	7.3	8.4	9.2			9.2
6	12.5	10.8	10.0	11.7		12.2	12.6	12.6
Mean	8.0	8.3	8.2					9.5
SD	2.6	2.1	1.7					2.6
				PO_2 (mmHg)				
1	61	60	61	59	58	60		60
2	64	60	70					70
3	63	67	56	56				56
4				64				64
5	48	45	49	49	50			50
6	60	63	62	60		54	49	49
Mean	59	59	60					58
SD	6	8	8					8
				PCO_2 (mmHg)				
1	50	49	51	50	51	49		49
2	44	41	42					42
3	42	38	39	42				42
4				43				43
5	60	60	56	55	58			58
6	35	34	36	36		36	37	37
Mean	46	44	45					45
SD	9	10	8					7
				pH				
1	7.38	7.39	7.38	7.39	7.38	7.40		7.40
2	7.38	7.38	7.38					7.38
3	7.38	7.39	7.40	7.38				7.38
4				7.37				7.37
5	7.36	7.34	7.37	7.38	7.38			7.38
6	7.45	7.45	7.44	7.44		7.44	7.44	7.44
Mean	7.39	7.39	7.39					7.39
SD	0.04	0.04	0.03					0.03

SPIROMETRY

Subject	Baseline	Placebo	Adenosine dose (mg/min)		
			2.3	4.3	Maximum
			FEV_1 (l)		
1	0.41	0.49	0.42	0.43	0.46
2		0.26	0.28	0.26	0.26
3	0.39	0.35	0.40	0.35	0.34
4	0.51	0.38	0.43	0.43	0.39
5	0.28	0.24	0.27	0.22	0.16
6	0.67	0.69	0.63	0.68	0.62
Mean	0.45	0.40	0.40	0.39	0.37
SD	0.15	0.17	0.13	0.16	0.16
			FVC (l)		
1	1.22	1.28	1.29	1.26	1.33
2		0.86	0.75	0.84	0.84
3	0.97	0.92	1.03	1.01	0.83
4	1.11	0.91	0.89	1.02	0.91
5	0.92	0.61	0.74	0.58	0.39
6	2.17	2.22	1.98	2.28	1.94
Mean	1.28	1.13	1.11	1.17	1.04
SD	0.51	0.57	0.47	0.59	0.53
			FEV_1/FVC (%)		
1	34.0	39.1	32.3	34.5	34.8
2		29.9	37.0	30.4	30.4
3	40.5	38.5	38.9	35.3	41.6
4	45.9	42.0	49.2	42.0	43.0
5	30.9	39.2	36.4	37.2	41.2
6	31.0	31.2	32.0	29.6	31.9
Mean	36.4	36.6	37.6	34.9	37.1
SD	6.6	4.9	6.3	4.6	5.5
			$FEF_{25-75\%}$ (l/s)		
1	0.20	0.21	0.17	0.18	0.19
2		0.09	0.12	0.11	0.11
3	0.16	0.17	0.17	0.16	0.16
4	0.32	0.17	0.21	0.19	0.18
5		0.07	0.10	0.08	0.06
6	0.24	0.21	0.24	0.23	0.19
Mean	0.23	0.15	0.17	0.16	0.15
SD	0.07	0.06	0.05	0.06	0.05

CHANGES IN FRC (l)

Subject	Time (min)					Mean of minutes 1 to 5
	1	2	3	4	5	
			Placebo infusion			
1	0.00	-0.07	0.04	-0.04	0.00	-0.01
2	-0.04	-0.04	-0.08	0.00	-0.02	-0.04
3	-0.03	-0.08	-0.20	-0.21	-0.23	-0.15
4	-0.10	-0.05	0.05	-0.03	0.08	-0.01
5	0.18	0.06	0.14	0.10	0.04	0.10
6	0.03	-0.06	-0.06	-0.03	0.03	-0.02
Mean	0.01	-0.04	-0.02	-0.03	-0.02	-0.02
SD	0.10	0.05	0.12	0.10	0.11	0.08
			Maximum dose of adenosine			
1	0.00	0.00	0.19	0.22	0.34	0.15
2	0.06	0.24	0.22			0.18
3	0.15	0.34	0.41			0.30
4	-0.03	0.25	0.29	0.41	0.34	0.25
5	0.24	0.28	0.28	0.24	0.22	0.25
6	0.05	0.54	0.67	0.72	0.77	0.55
Mean	0.08	0.27	0.34			0.28
SD	0.10	0.17	0.18			0.14

APPENDIX 7 - PUBLICATIONS FROM THE THESIS

The following publications have arisen from the work of this thesis:

Reid PG, Watt AH, Routledge PA & Smith AP (1987). Intravenous infusion of adenosine but not inosine stimulates respiration in man. Br J Clin Pharmacol 23:331-8.

Watt AH, Reid PG, Stephens MR & Routledge PA (1987). Adenosine-induced respiratory stimulation in man depends on site of infusion. Evidence for an action on the carotid body? Br J Clin Pharmacol 23:486-90.

Reid PG, Fraser AG, Watt AH, Henderson AH & Routledge PA (1990). Acute haemodynamic effects of intravenous infusion of adenosine in conscious man. Eur Heart J 11:1018-28.

Reid PG, Watt AH, Penny WJ, Newby AC, Smith AP & Routledge PA (1991). Plasma adenosine concentrations during adenosine-induced respiratory stimulation in man. Eur J Clin Pharmacol 40 (in press).

Br. J. clin. Pharmac. (1987), **23**, 331–338

Intravenous infusion of adenosine but not inosine stimulates respiration in man

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1 The effects on respiration of intravenous infusions of the endogenous nucleoside adenosine and its deaminated metabolite, inosine, administered in random order, single-blind, were compared in six healthy volunteers.

2 The infusion rate of each nucleoside was initially 3.1 mg min^{-1} and was increased stepwise every 2 min, as tolerated, up to a possible maximum of 23.4 mg ml^{-1}. The maximum dose rates received by all subjects were 8.5 mg min^{-1} for adenosine and 16.8 mg min^{-1} for inosine.

3 Adenosine infusion at rates of 6.1 mg min^{-1} and above caused a significant increase in minute ventilation, principally due to an increase in tidal volume, with an associated significant fall in end-tidal P_{CO_2}. Mean inspiratory flow rate increased and expiratory duration decreased during adenosine infusion, but there was no change in inspiratory duration.

4 Adenosine infusion also caused a significant increase in heart rate and a slight, but significant increase in systolic blood pressure.

5 Infusion of inosine at dose rates up to 16.8 mg min^{-1} produced no pharmacological effects.

6 This study shows that adenosine by infusion produces sustained respiratory stimulation in man and demonstrates that it does not depend on prior conversion of adenosine to inosine or related metabolites and that it is not secondary to systemic hypotension.

Keywords adenosine inosine respiration

Introduction

The endogenous nucleoside adenosine exerts a variety of physiological and pharmacological effects (Lancet, 1985). Recently, dose dependent stimulation of respiration in man in association with biphasic heart rate changes produced by intravenous boluses of adenosine has been described (Watt & Routledge, 1985, 1986). Adenosine is rapidly metabolised, either by re-incorporation into the nucleotide pool or by degradation initially to inosine (Klabunde, 1983) and has an *in vitro* half-life in human whole blood of less than 10 s (Klabunde, 1983). Significant metabolism of adenosine would therefore be expected during the observed 15–20 s interval between injection of adenosine and the onset of respiratory stimulation. It is therefore unclear whether the effects on respiration observed were due to adenosine itself or a metabolite.

Other effects of adenosine include hypotension when administered in large doses (Sollevi *et al.*,

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1984) and this itself might cause respiratory stimulation.

In this study we compared the effects on respiration, heart rate and blood pressure of intravenous infusions of adenosine and its metabolite inosine in order to clarify the mechanism of the previously reported respiratory stimulation produced by adenosine and to investigate whether the transitory effects seen after intravenous bolus doses of adenosine could be sustained by continuous infusion of the drug.

Methods

Eight healthy volunteers (seven male) aged 23 to 33 years gave informed, written consent to participate in the study which was approved by the Hospital Ethics Committee. All subjects were asked to abstain from caffeine-containing beverages for at least 12 h prior to the study. Adenosine and inosine were administered in random order, single-blind, by intravenous infusions separated by 30 min. This interval was chosen on the basis of our finding in a pilot study that the cardiorespiratory effects produced by adenosine infusion resolve within 1 to 2 min of stopping the infusion. The infusion rate of each nucleoside was initially 3.1 mg min^{-1} and was increased every 2 min up to a possible maximum of 23.4 mg min^{-1} (maximum possible number of stages: 7). Each infusion was discontinued following administration of a dose of 23.4 mg min^{-1} or earlier at the request of a subject. The maximum dose rates received ranged from 8.5 to 23.4 mg min^{-1} for adenosine and 16.8 to 23.4 mg min^{-1} for inosine.

The electrocardiogram (ECG) was monitored throughout each infusion. Recordings of the ECG and measurements of blood pressure (using an Accoson mercury sphygmomanometer, taking phase V as diastolic) were made at baseline and at 1 min intervals throughout each infusion. A respiratory trace was obtained from a Lectromed type 4320 respiration transducer (calibrated by a spirometer) secured around the chest. We have found this to give a linear response to increasing tidal volume in supine subjects. P_{CO_2} was measured continuously in gas sampled by a catheter, whose tip was clipped to the upper front teeth, using a PK Morgan Ltd. Type 901 MK.2 high speed response CO_2 analyser. The respiratory and P_{CO_2} traces were recorded on an Ormed MX 216 recorder. Respiratory variables (respiratory rate, tidal volume, minute ventilation, inspiratory duration, expiratory duration and total breath duration) and end-tidal P_{CO_2} were subsequently derived from the traces obtained. Subjects were asked to report any subjective sensations at 1 min intervals and were aware that an infusion would be stopped immediately at their request. In four subjects spirometry was performed prior to and immediately after each infusion using a Micromedical Instruments Pocket Spirometer (Chowienczyk & Lawson, 1982).

The solutions used were sterile preparations of adenosine (Sigma) or inosine (Sigma) in 0.9% sodium chloride at concentrations of 5 mg ml^{-1}. Infusion rates were regulated using a Harvard infusion pump, model 2681.

Comparisons of respiratory rate, tidal volume, minute ventilation, end-tidal P_{CO_2}, heart rate and blood pressure at different infusion rates, up to 8.5 mg min^{-1} for adenosine and 16.8 mg min^{-1} for inosine, were made using two-way analysis of variance and Student Newman-Keuls test. In one subject the study was stopped because of occipital headache and in one subject an inadequate respiratory trace was obtained. Data were therefore analysed for six subjects. Student's paired *t*-test was used to compare baseline values of the above variables as well as mean inspiratory flow, inspiratory duration, expiratory duration and inspiratory duration over total breath duration (T_I/T_{Tot}) with values at the maximum dose of each nucleoside tolerated, and to compare spirometric variables (peak expiratory flow rate (PFR), forced expiratory volume in 1 s (FEV_1) and forced vital capacity (FVC)) before and after each infusion.

Results

The effects of adenosine infusion on respiratory rate, tidal volume, minute ventilation and end-tidal P_{CO_2} and on heart rate and blood pressure are shown in Figures 1 and 2 respectively. All subjects received up to 8.5 mg min^{-1} of adenosine but the maximum infusion rate tolerated ranged from 8.5 to 23.1 mg min^{-1} (mean ± s.d.: 13.5 ± 5.7 mg min^{-1}). Data are therefore presented for infusion rates up to 8.5 mg min^{-1} and for the maximum infusion rate received by each subject.

Minute ventilation increased significantly during adenosine infusion from 6.6 ± 4.5 l min^{-1} at baseline to 13.0 ± 5.7 l min^{-1} at an infusion rate at 8.5 mg min^{-1}, and 19.2 ± 11.2 l min^{-1} at the maximum infusion rate ($P < 0.001$ and $P < 0.005$ respectively). These changes were predominantly due to an increase in tidal volume from 0.5 ± 0.4 l at baseline to 0.9 ± 0.4 l during adenosine infusion at 8.5 mg min^{-1}, and 1.2 ± 0.6 l at the maximum infusion rate ($P < 0.001$ and $P < 0.05$ respectively).

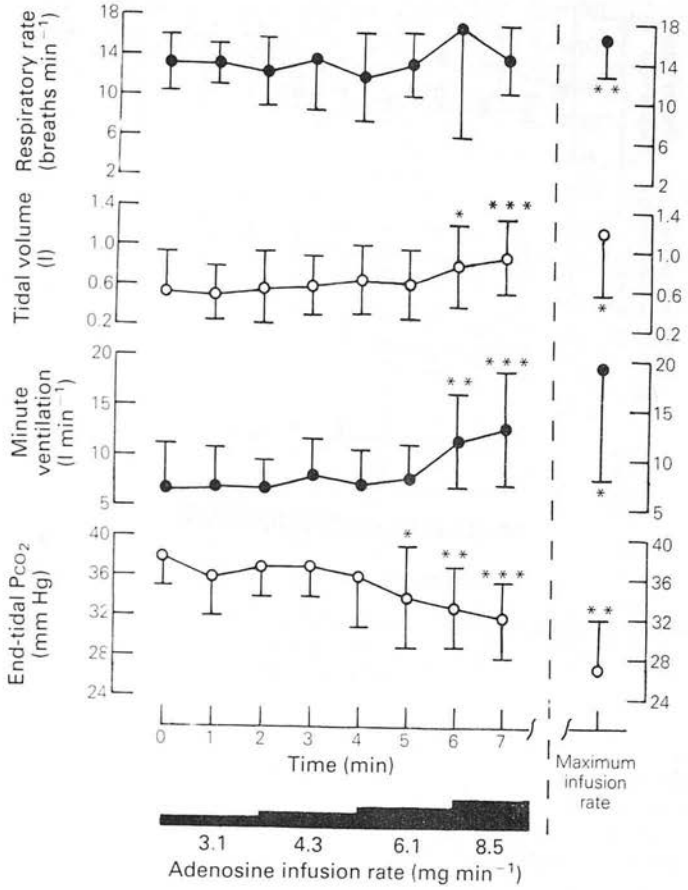

Figure 1 Respiratory rate, tidal volume, minute ventilation and end-tidal P_{CO_2} during adenosine infusion. Data are shown as mean ± s.d., $n = 6$ except for 3.1 mg min^{-1} where $n = 5$. For explanation of maximum dose please see text.
$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ for comparisons with baseline.

The changes in respiratory rate were only significant at the maximum infusion rate (16 ± 4 breaths min^{-1} *vs* 13 ± 3 breaths min^{-1} at baseline; $P < 0.01$).

The increase in ventilation during adenosine infusion was accompanied by an increase in mean inspiratory flow (tidal volume/inspiratory duration) from 16.8 ± 9.6 l min^{-1} at baseline to 46.8 ± 21.0 l min^{-1} at the maximum infusion rate ($P < 0.05$). The expiratory duration fell from 3.1 ± 1.0 s at baseline to 2.4 ± 0.9 s at the maximum infusion rate ($P < 0.01$) but there was no change in inspiratory duration (1.7 ± 0.5 s at baseline *vs* 1.5 ± 0.5 s at the maximum infusion rate; $0.1 < P < 0.2$) or T_I/T_{Tot} (0.4 ± 0.1 at baseline and at the maximum infusion rate).

End-tidal P_{CO_2} fell significantly during adenosine infusion from 38 ± 3 mm Hg at baseline to 32 ± 4 mm Hg at an infusion rate of 8.5 mg min^{-1}, and 27 ± 5 mm Hg at the maximum infusion rate ($P < 0.001$ and $P < 0.01$ respectively).

Heart rate increased during adenosine infusion from 67 ± 7 beats min^{-1} at baseline to 86 ± 15 beats min^{-1} at an infusion rate of 8.5 mg min^{-1}, and 105 ± 9 beats min^{-1} at the maximum infusion rate ($P < 0.01$ and $P < 0.001$ respectively). One subject developed a transient bradycardia of 30 min^{-1} with second degree heart block for a few beats during breath-holding immediately after discontinuing adenosine infusion at a rate of 16.8 mg min^{-1}. Rhythm in this subject quickly reverted to sinus tachycardia. In no other subject was a bradycardia seen.

Systolic blood pressure increased significantly during adenosine infusion from 120 ± 10 mm Hg at baseline to 131 ± 11 mm Hg at an infusion rate

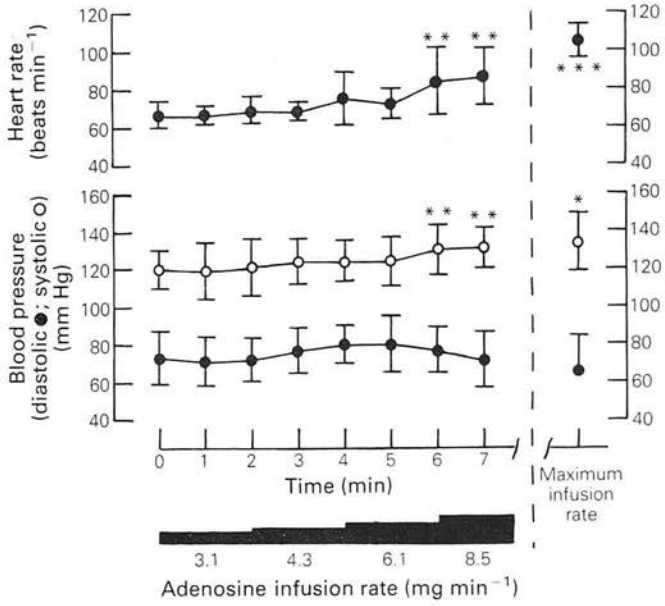

Figure 2 Heart rate and blood pressure during adenosine infusion. Data are shown as mean ± s.d., $n = 6$ except for 3.1 mg min^{-1} where $n = 5$. For explanation of maximum dose please see text.
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ for comparisons with baseline.

of 8.5 mg min^{-1}, and 134 ± ± 15 mm Hg at the maximum infusion rate ($P < 0.01$ and $P < 0.05$ respectively). Diastolic blood pressure changed biphasically increasing from 73 ± 14 mm Hg at baseline to 80 ± 10 mm Hg during adenosine infusion at 4.3 mg min^{-1}, with a fall at higher infusion rates, to 66 ± 19 mm Hg at the maximum infusion rate. These changes were, however, not statistically significant ($P > 0.2$). Significant hypotension did not occur during adenosine infusion at any of the doses used in this study.

In contrast to the changes observed during adenosine infusion, no significant changes in the above variables were observed during inosine infusion, despite the higher maximum infusion rate (16.8 mg min^{-1}) received by all subjects. Respiratory rate, tidal volume and minute ventilation during inosine infusion are shown in Figure 3 and heart rate and blood pressure in Figure 4. Results for mean respiratory flow, inspiratory duration, expiratory duration, and T_I/T_{Tot} (baseline *vs* infusion at 16.75 mg min^{-1} were 21.0 ± 9.6 *vs* 20.4 ± 7.2 l min^{-1}, 1.5 ± 0.6 *vs* 1.7 ± 0.6 s, 3.4 ± 0.7 *vs* 3.0 ± 1.3 s, and 0.3 ± 0.1 *vs* 0.4 ± 0.1 respectively ($P > 0.2$ for all comparisons).

During adenosine infusion facial flushing was reported by all eight subjects, dyspnoea by seven, throat discomfort by five, epigastric discomfort by four, headache by four and retrosternal discomfort by two. One subject was able to tolerate the maximum dose of adenosine infused (23.4 mg min^{-1}). In all other subjects the infusion was stopped at a lower dose rate because of the degree of dyspnoea and other sensations experienced. Three subjects experienced paraesthesiae in the hands at the end of the study. All sensations resolved within 1 to 2 min after stopping the infusion.

During inosine infusion one subject reported lightheadedness but all other subjects were asymptomatic.

No subject reported wheeziness and spirometry showed no significant changes in the four subjects tested. In this group FEV_1, PEFR and FVC (best of three readings; mean ± s.d.) were 4.25 (± 0.50) l, 635 (± 65) l min^{-1} and 5.24 (± 0.63) l respectively at baseline and 4.10 (± 0.47) l, 594 (± 48) l min^{-1} and 5.27 (± 0.56) l respectively immediately following adenosine infusion. Values for FEV_1, PFR and FVC prior to inosine infusion were 4.12 (± 0.45) l, 572 (± 60) l min^{-1} and 5.20 (± 0.72) l respectively, and immediately following inosine infusion were 4.19 (± 0.49) l, 588 (± 73) l min^{-1} and 5.30 (± 0.63) l respectively.

Discussion

This study confirms the respiratory stimulant property of adenosine in man, which was first

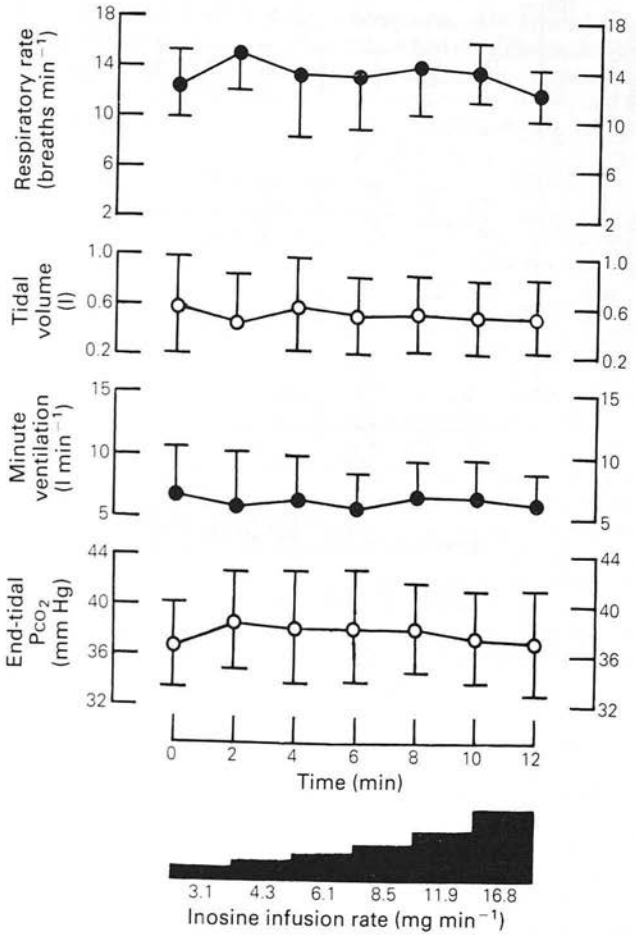

Figure 3 Respiratory rate, tidal volume, minute ventilation and end-tidal P_{CO_2} during inosine infusion. Data are shown as mean ± s.d.

demonstrated as a transient effect following intravenous boluses of adenosine (Watt & Routledge, 1985), and shows that respiratory stimulation is sustained during infusion of the nucleoside. Similar findings have recently been reported in abstract form by Biaggioni *et al.* (1986).

Adenosine is rapidly removed from the circulation principally by cellular uptake and then either metabolised by deamination to inosine or reincorporated into the nucleotide pool (Klabunde, 1983). The present study has shown that intravenous inosine is without effect on respiration in the dose range studied and therefore the respiratory stimulation produced by adenosine in this dose range does not depend on prior metabolism to inosine.

Our data do not exclude the possibility that phosphorylation of adenosine is a prerequisite for its respiratory stimulant effect. However, it has been shown in the cat that adenosine increases neural discharges from the carotid body (McQueen & Ribeiro, 1981), whereas a stable analogue of adenosine triphosphate (ATP) was without effect (McQueen & Ribeiro, 1983). In addition studies using long acting analogues of adenosine suggest that it acts via cell surface receptors of the A2 subtype in the carotid body (Ribeiro & McQueen, 1986).

Mean inspiratory flow can provide an index of 'inspiratory drive' provided the mechanical properties of the respiratory system are fixed (Remmers, 1976). The changes observed in this study during adenosine infusion, namely an increase in mean inspiratory flow and a reduction in expiratory duration are qualitatively similar to those produced by a number of respiratory stimuli, including hypoxia and hypercapnia

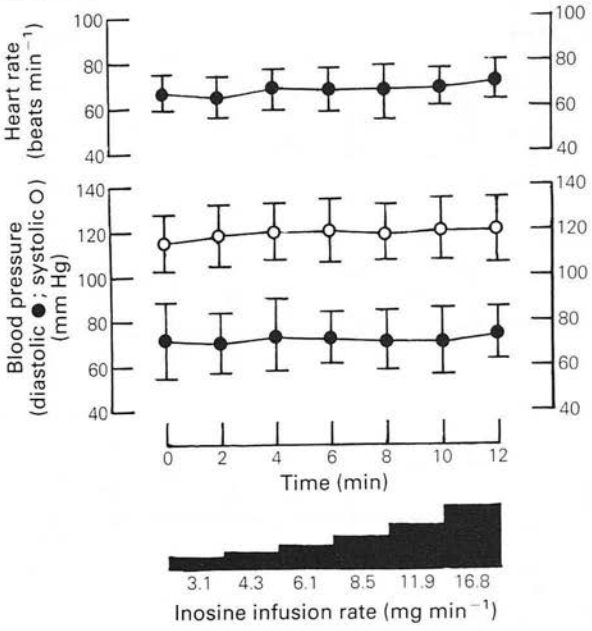

Figure 4 Heart rate and blood pressure during inosine infusion. Data are shown as mean ± s.d.

(Remmers, 1976). We found no change in inspiratory duration during adenosine infusion. It has been suggested that whereas inspiratory duration may remain constant during hypercapnia until tidal volume is increased to 3–5 times the resting value, there is a progressive shortening of inspiratory duration during progressive isocapnic hypoxia (Rebuck *et al.*, 1976). Others, however, have found no change in inspiratory duration during ventilatory stimulation by hypoxia, hypercapnia and exercise (Cunningham & Gardner, 1972; Jennett *et al.*, 1974). In any case a reduction in inspiratory duration when it occurs is considerably less than the reduction in expiratory duration.

The present results together with our finding in man that perfusion of the carotid circulation by adenosine-rich blood is necessary to produce respiratory stimulation (Watt *et al.*, 1986) and the findings of Dixon *et al.* (1986) that adenosine potentiates ventilatory responses to hypoxia but not hypercapnia in man are consistent with the hypothesis that adenosine stimulates respiration by an action in the carotid body. This is supported by findings in the rabbit (Buss *et al.*, 1986) and the rat (Monteiro & Ribeiro, 1986) that stimulation of respiration by adenosine is abolished by section of the nerve supply to the carotid body and the finding in a number of species that various adenosine analogues act centrally as respiratory depressants (Hedner *et al.*, 1982; Eldridge *et al.*, 1984 and Wessberg *et al.*, 1985). Studies using adenosine antagonists at cell surface receptors, e.g. aminophylline, or inhibitors of nucleoside transport e.g. dipyridamole, would further characterise the mechanism(s) involved.

Adenosine acts as a vasodilator in several vascular beds (Berne, 1980; Berne *et al.*, 1974; Proctor, 1984) and both ATP, which is rapidly hydrolysed to adenosine *in vivo*, and adenosine have been used as hypotensive agents in man (Fukunaga *et al.*, 1982; Sollevi *et al.*, 1984). In the present study hypotension was not an effect of adenosine infusion, but on the contrary a mild, but statistically significant increase in systolic blood pressure was observed. The observed respiratory stimulation is therefore not attributable to hypotension which has only been reported during adenosine infusion to anaesthetised subjects who were pretreated with dipyridamole (Sollevi *et al.*, 1984). A rise in systolic blood pressure in association with a decreased diastolic blood pressure and increased heart rate has been reported by others (Biaggioni *et al.*, 1985) who found plasma noradrenaline and adrenaline levels to be elevated during adenosine infusion. The pressor effect of adenosine may represent a non-specific sympathetic response to the subjective sensations experienced or alternatively may be secondary to stimulation of the carotid body which has been described in animals (Daly & Scott, 1962). Further studies

are necessary to clarify the mechanism(s) of the observed blood pressure changes.

Adenosine administered by intravenous bolus produces a biphasic heart rate response: an initial transient bradycardia followed by a more sustained tachycardia (Watt & Routledge, 1986). The initial bradycardia is seen in isolated hearts and probably represents a direct negative chronotropic effect of adenosine on the sinoatrial and atrioventricular nodes (Szentmiklosi *et al.*, 1980). In the present study only an increase in heart rate was seen during adenosine infusion, as has been reported by others (Biaggioni *et al.*, 1985). The mechanism of this requires elucidation. In dogs a tachycardia following carotid body stimulation has been seen as a reflex secondary to increased ventilation (Daly & Scott, 1958). A similar mechanism may in part explain the heart rate changes seen in the present study. Negative chronotropic effects of intravenous adenosine boluses are probably exerted in the heart before other responses that may have a positive chronotropic effect, e.g. carotid body stimulation, can begin to take effect. Absence of a 'bolus effect' may explain why only an increase in heart rate was seen in this study using an intravenous infusion of adenosine.

Inhaled adenosine causes bronchonstriction in asthmatics, but not normal subjects (Cushley *et al.*, 1983). The mechanism is unclear (Cushley & Holgate, 1985). In the rat intravenous adenosine has been found to cause bronchoconstriction (Pauwels & Van Der Straeten, 1983). In the present study no subject reported a sensation of wheeze and in the four subjects in whom spirometry was performed there were no significant changes. Biaggioni *et al.* (1986) observed no change in spirometry in 12 subjects receiving adenosine infusion. Unlike the bronchoconstriction produced by inhaled adenosine in asthmatics, which had not fully abated within 30 min (Cushley *et al.*, 1983), the increased respiration produced by intravenous adenosine in the present study was observed to resolve within 1 min. These observations suggest that the respiratory stimulation is not secondary to airflow limitation. Further studies are necessary to examine the effects of intravenous adenosine on airway calibre in normal subjects and those with reversible airways obstruction.

The finding that intravenous adenosine infusions stimulate respiration raises two important questions: (1) Does adenosine have a physiological role in the control of respiration, possibly by mediating the ventilatory response to hypoxia within the carotid body, as has been suggested (Watt & Routledge, 1985)? (2) Can the respiratory stimulant property of intravenous adenosine be usefully applied, e.g. in patients with respiratory failure? With regard to a potential therapeutic role for adenosine, the adverse subjective sensations we have noted in this study in which we examined the dose-response relationship between ventilation and adenosine infusion rate up to the limit of each subject's tolerance may not be relevant to longer term use of lower dose infusion of the nucleoside. If adenosine were to adversely affect airway calibre, this may be a limiting factor in patients with airways obstruction. Further studies are required to answer these questions.

A.H.W. is supported by the Welsh Scheme for the Development of Health and Social Research. We thank the Sterile Products Unit, Pharmacy Department, University Hospital of Wales for supplying the sterile preparations of adenosine and inosine. We are grateful to Mrs Sue Forster for typing the manuscript.

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(Received 11 July 1986, accepted 16 October 1986)

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(Received 3 September 1986, accepted 14 November 1986)

Br. J. clin. Pharmac. (1987), **23**, 486–490

Adenosine-induced respiratory stimulation in man depends on site of infusion. Evidence for an action on the carotid body?

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Adenosine is an endogenous nucleoside which stimulates respiration in man and other mammals. In animals adenosine appears to initiate respiratory stimulation within the carotid body, but whether this is the site of action in man is not known. We administered adenosine by intra-aortic infusion to 12 subjects undergoing cardiac catheterisation. When adenosine was infused at three sites proximal to the carotid circulation, minute ventilation was significantly higher than baseline values or those during adenosine infusion at a more distal site. These results support the hypothesis that adenosine-induced respiratory stimulation in man is mediated in the carotid body.

Introduction

Adenosine is an endogenous nucleoside which exerts various pharmacological effects (Berne, 1980; Lancet, 1985; Newby, 1984), many of which appear to be related to the balance between energy (or oxygen) supply and demand (Newby, 1984).

We recently identified a dose-related respiratory stimulant effect of adenosine in man (Watt & Routledge, 1985), and suggested that this effect was likely to be carotid body mediated and that it might be relevant to the ventilatory stimulation produced by hypoxia which is carotid body dependent in man and some other mammals (Chalmers *et al.*, 1967; Lugliani *et al.*, 1971).

Adenosine has a half-life in human blood of less than 10 s (Klabunde, 1983). Therefore if adenosine-induced respiratory stimulation in man is carotid body mediated then it would be expected that administration of adenosine proximal to the carotid circulation would stimulate respiration whereas more distal administration of the nucleoside would have no such effect. We examined this hypothesis in 12 patients undergoing cardiac catheterisation.

Methods

Informed, written consent was obtained from 12 patients (10 male, aged 55 ± 7 years) scheduled to undergo cardiac catheterisation on clinical grounds for investigation of chest pain. Adenosine was administered according to a protocol approved by the hospital ethics committee. Careful consideration was given to the possibility of adverse cardiac events in such patients but it was considered that clinically significant adverse events were unlikely in view of the short half-life of adenosine, less than 10 s (Klabunde, 1983), and the ease of catheter withdrawal to a more distal part of the aorta.

Adenosine was administered by continuous intra-aortic infusion sequentially at five sites: (1) immediately above the aortic valve, (2) mid-ascending thoracic aorta, (3) top of the aortic arch, (4) mid-descending thoracic aorta and (5) just proximal to the top of the aortic arch (see Figure 1). Sites 1, 2 and 5 are proximal to the carotid circulation, site 3 is close to the origin of those vessels, and site 4 is situated distal to the carotid vessels. The initial rate of intra-aortic adenosine infusion was derived from our experi-

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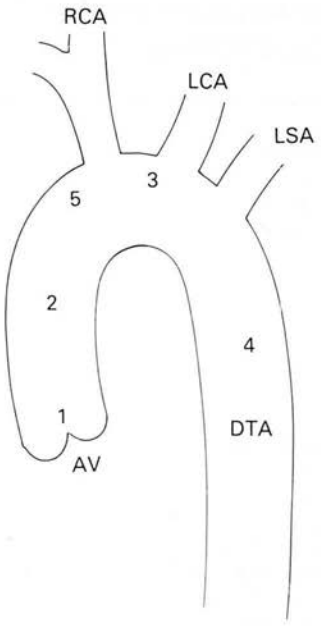

Figure 1 Schematic diagam showing infusion sites in the thoracic aorta in relation to the origin of the carotid circulation. Right common carotid artery (RCA), left common carotid artery (LCA), left subclavian artery (LSA), descending thoracic aorta (DTA) and aortic valve (AV).

ence of intravenous adenosine infusion: an intravenous infusion rate of 6 mg min^{-1} produced slight respiratory stimulation in man (Reid *et al.*, 1987). We assumed a half-life of adenosine in blood of about 10 s (Klabunde, 1983) and a circulation time of 20 s (from an antecubital vein). The infusion rate at site 1 was therefore initially 1.58 mg min^{-1}, and was increased as necessary to a level which produced a moderate increase in minute ventilation. In this group of patients the final dose was 2.86 ± 0.97 mg min^{-1} (mean ± s.d.), and the infusion was continued at a constant rate throughout the remainder of the study, as the cardiac catheter was moved at 1 min intervals from one site to the next. Patients were 'blind' to the timing and direction of catheter movements.

An electrocardiogram was recorded throughout the study. A respiratory trace was recorded at 1 min intervals from a Lectromed type 4320 respiration transducer (which had been calibrated against a spirometer) on an Ormed MX216 recorder. Minute ventilation was calculated from the product of tidal volume and respiratory rate. Intra-aortic pressure was measured in eight subjects via the cardiac catheter before and immediately after the adenosine infusion. In four patients right femoral artery pressure was measured at 1 min intervals from a side-arm of a sheath through which the cardiac catheter had been inserted. Patients were asked at 1 min intervals to report any subjective sensations.

The adenosine solution used was a sterile preparation of adenosine (Sigma) in 0.9% sodium chloride at a final adenosine concentration of 5 mg ml^{-1}. Infusion rates were controlled using a Harvard model 2681 infusion pump.

Eight patients were taking oral β-adrenoceptor blockers, seven oral or dermal nitrate preparations and five oral calcium antagonists. One patient was receiving amiodarone, one bendrofluazide, and one frusemide (20 mg daily). All therapy, other than sublingual nitrates, was stopped at least 12 h prior to the study.

Comparisons of log-transformed minute ventilation, heart rate and blood pressure at different sites were made using two-way analysis of variance and Student Newman-Keuls test. Blood pressure before and after the adenosine infusion was compared using Student's paired *t*-test.

Results

Respiratory, heart rate and blood pressure data are summarised in Table 1. In one patient the trace recorded at position 4 could not be measured and in one patient the study was stopped prematurely because of epigastric discomfort. Data were therefore analysed for the remaining 10 patients.

There was a significant difference in minute ventilation at different infusion sites ($F = 5.444$, Num d.f. = 5, Den d.f. = 45, $P < 0.002$). Minute ventilation at baseline and site 4 did not differ from each other ($Q = 0.989$, $P > 0.50$) but were significantly less than minute ventilation at sites 1, 2 or 5 ($P < 0.025$ for each comparison). Minute ventilation at site 3 was intermediate and did not differ significantly from that at any other site. There were no other significant inter-site differences in minute ventilation.

The difference in minute ventilation was attributable to inter-site differences in tidal volume ($F = 3.886$, Num d.f. = 5, Den d.f. = 45, $P < 0.02$). Tidal volume during adenosine infusion at sites 1, 2 and 5 was higher than at baseline ($P < 0.05$ for each comparison). Tidal volume at site 4 did not differ from baseline ($Q = 0.711$, $P > 0.50$), but approached a significant difference from sites 1, 2 and 5 ($0.05 < P < 0.10$ for each comparison). There was no difference in respiratory rate at different infusion sites ($F = 1.482$, Num d.f. = 5, Den d.f. = 45, $P > 0.20$).

Table 1 Respiration, heart rate and mean blood pressure during adenosine infusion at the sites described. Data are shown as mean ± s.d.

Variable	n	*Baseline*	*Site 1*	*Site 2*	*Site 3*	*Site 4*	*Site 5*
Minute ventilation ($l\ min^{-1}$)	10	5.31 ± 1.68	8.78 ± 3.53	10.13 ± 8.93	6.83 ± 2.26	5.05 ± 2.53	9.71 ± 6.21
Respiratory rate (min^{-1})	10	16.1 ± 3.6	16.3 ± 3.6	16.6 ± 5.0	15.1 ± 2.4	13.8 ± 3.4	16.7 ± 3.0
Tidal volume (l)	10	0.35 ± 0.14	0.57 ± 0.29	0.60 ± 0.34	0.46 ± 0.18	0.38 ± 0.18	0.62 ± 0.45
Heart rate (beats min^{-1})	10	59 ± 10	63 ± 12	68 ± 14	70 ± 13	69 ± 14	73 ± 15
Mean right femoral artery pressure (mm Hg)	4	92 ± 10	94 ± 16	99 ± 12	94 ± 14	92 ± 12	102 ± 13

There was also a significant difference in heart rate at different infusion sites ($F = 13.974$, Num d.f. = 5, Den d.f. = 45, $P < 0.001$). Heart rate at baseline and at site 1 was significantly less ($P < 0.01$ for each comparison) than heart rate at sites 2, 3, 4 or 5 which were not significantly different from one another.

In the eight patients in whom mean intra-aortic pressure was measured before (89 ± 8 mm Hg) and immediately after (84 ± 12 mm Hg) adenosine infusion, there was no significant change ($t = 1.754$, d.f. = 7, $P > 0.10$). In the four patients in whom right femoral artery pressure was measured at each infusion site no significant changes were demonstrable ($F = 1.054$, Num d.f. = 5, Den d.f. = 15, $P > 0.50$).

Adverse effects were reported by a number of patients during adenosine infusion but these were mild with one exception. That patient, who had a symptomatic hiatus hernia, experienced epigastric pain during adenosine infusion at site 4 and the study was promptly terminated at the patient's request. While no other subject requested that the study be terminated (although all were aware that they might do so) eight reported dyspnoea, eight had chest discomfort (six at site 4), eight experienced epigastric discomfort (one at site 1, six at site 4), two reported nausea, six mentioned facial flushing and four described discomfort in the neck or throat. All subjective sensations resolved within 60 s of stopping the adenosine infusion.

Discussion

The present study confirms the respiratory-stimulant effect of adenosine in man which we reported previously (Watt & Routledge, 1985; Reid *et al.*, 1987). Further the results demonstrate that the respiratory-stimulant effect of intravenous adenosine depends on the site of administration. Adenosine infused distal to the carotid circulation (site 4) caused no respiratory stimulation, but infusion of adenosine proximal to the carotid circulation caused a respiratory stimulation both before (sites 1 and 2) and after (site 5) more distal adenosine infusion.

These findings raise the question of why adenosine-induced respiratory stimulation should depend on perfusion of the carotid circulation. The effect could be mediated by the carotid bodies or brain chemoreceptors. The latter alternative appears unlikely because adenosine analogues depress respiration when applied locally to the brain (Eldridge *et al.*, 1984; Mueller *et al.*, 1984). We found that adenosine-induced respiratory stimulation in the rabbit was abolished by bilateral division of the afferent nerve supply to the carotid bodies (Buss *et al.*, 1986) and others have demonstrated the same finding in the rat (Monteiro & Ribeiro, 1986). This suggests that adenosine stimulates respiration by an action on the carotid body in those species. The data presented above support but do not prove the hypothesis that adenosine-induced respiratory stimulation in man is also carotid body mediated.

The pattern of site-dependent changes in heart rate differed from that of minute ventilation. Heart rate increased after adenosine infusion was started; the increase over baseline values being apparent during infusion at sites 2, 3, 4 and 5. We did not identify any fall in blood pressure to account for the changes in heart rate. It is not clear why heart rate did not increase at site 1, but it may partly be because a sufficiently high concentration of adenosine might be per-

fusing the coronary circulation, to exert a negative chromotropic effect on the sinoatrial node (Szentmiklosi *et al.*, 1980) to oppose any increase in heart rate produced by other mechanisms. The increase in heart rate seen at other sites may be a reflex, perhaps secondary to the increase in ventilation produced by carotid body stimulation, as has been observed in the dog (Daly & Scott, 1958). If this is the case it is unclear why the changes in heart rate show a pattern different from the respiratory changes. Heart rate does not return rapidly to baseline following boluses of adenosine (see figure in Watt & Routledge, 1986) so it is possible that in the present study there was insufficient time during the period of infusion at site 4 for a return to baseline heart rate to occur. Alternatively a sympathetic reflex secondary to the adverse effects experienced may have contributed to the tachycardia and hence its persistence during infusion at site 4 where some adverse effects were more common. Evidence of sympathetic stimulation by adenosine has been provided by Biaggioni *et al.* (1985) who reported elevated plasma adrenaline and noradrenaline levels in association with increased heart rate and systolic blood pressure during peripheral infusion of adenosine. The mechanism of this sympathetic activation remains to be elucidated.

Eight of the 10 patients were receiving treatment with β-adrenoceptor blockers and this is likely to have reduced the magnitude of the heart rate changes in those patients. In the two patients who were not receiving β-adrenoceptor blockers heart rate increased by up to 34 beats min^{-1}, while in the β-adrenoceptor blocked patients heart rate increased by up to 19 beats min^{-1} from baseline.

Intravenous adenosine in high doses is known to lower blood pressure in anaesthetised man (Sollevi *et al.*, 1984) and in laboratory mammals (Fukunaga *et al.*, 1982). At the doses used in the present study no changes in arterial blood pressure were detected. Numbers were small, therefore a Type II error is a possibility but in other studies using intravenous adenosine infusion we and others observed respiratory stimulation without hypotension and with an increase in systolic blood pressure (Reid *et al.*, 1987; Biaggioni *et al.*, 1986). Therefore it is unlikely that hypotension is a necessary factor contributing to the observed adenosine-induced respiratory stimulation.

The occurrence of chest discomfort in some patients during intra-aortic infusion is of interest in the context of the adenosine-induced retrosternal discomfort reported recently by Sylven *et al.* (1986). They proposed that exogenous adenosine produced such transient chest discomfort by directly stimulating afferent cardiac nerves, and further proposed that adenosine released spontaneously during cardiac ischaemia contributes to the pain of angina pectoris. They provided no information as to changes in coronary flow associated with such sensations or whether such sensations are ever cardiac in origin. We have shown that adenosine increases coronary flow in man (Watt *et al.*, 1986) and in 9 of those 10 patients transient retrosternal discomfort occurred in association with doubled coronary flow. Thus retrosternal discomfort is not attributable in those patients with normal coronary arteries to a fall in coronary blood flow. The present study provides information on the site of origin of adenosine-induced chest discomfort. In seven of the eight patients reporting such discomfort, it occurred during adenosine infusion at sites 3 and 4. When one considers that the half-life of adenosine in human blood is less than 10 s (Klabunde, 1983), it is unlikely that such chest sensations in those patients are cardiac in origin. In the single patient who reported 'chest tightness' during adenosine infusion at site 1 but at no other site, the heart might appear to be the source of adenosine-induced discomfort. That patient had a 50% left anterior descending coronary artery stenosis. Coronary flow increases distal to stenoses of that degree in animal hearts in response to vasodilators (Knabb *et al.*, 1985) so a fall in absolute coronary flow distal to the stenosis is unlikely in that patient. The occurrence of 'chest tightness' in that patient would therefore appear to provide support for a possible cardiac origin of adenosine-induced retrosternal discomfort in some circumstances.

Adenosine reproduces the epigastric pain of duodenal ulceration (Watt *et al.*, 1987). While epigastric discomfort of some degree occurred in 8 of 10 patients in this study, in only the patient with a hiatus hernia was the discomfort marked. It may be, therefore, that not only duodenal ulceration but also other inflammatory lesions of the upper gastrointestinal tract may predispose to adenosine-induced epigastric discomfort by mechanisms as yet undefined.

We previously suggested that adenosine stimulates respiration in man by an action on the carotid body (Watt & Routledge, 1985). The results of the present study support this suggestion and are consonant with the data supporting the carotid body as being the site of adenosine-induced respiratory stimulation in the rabbit (Buss *et al.*, 1986) and rat (Monteiro & Ribeiro, 1986). The ventilatory response to hypoxia in man is also carotid body dependent (Lugliani *et al.*, 1971). It remains to be established whether

adenosine plays a role in the mechanisms by which the ventilatory response to hypoxia is initiated in the carotid body.

A.H.W. was supported by the Welsh Scheme for the Development of Health and Social Research. We thank the Sterile Products Unit, Pharmacy Department, University Hospital of Wales for supplying the sterile preparation of adenosine. We are grateful to Mrs Sue Forster for typing the manuscript.

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(Received 21 October 1986, accepted 9 December 1986)

European Heart Journal (1990) **11**, 1018–1028

Acute haemodynamic effects of intravenous infusion of adenosine in conscious man

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KEY WORDS: Adenosine, myocardial contraction, pulmonary circulation, respiration, vasodilatc agents.

The acute haemodynamic effects of intravenous infusion of adenosine, a dilator of most vascular beds, wer studied in 16 patients (seven with coronary artery disease, nine with normal coronary arteries) undergoin cardiac catheterization for investigation of chest pain.

At the lowest dose used (4·3 mg min^{-1}) adenosine increased minute ventilation by 44% ($P<0·01$, $n=11$ and reduced pulmonary vascular resistance by 20% ($P<0·05$) without causing other significant haemo dynamic changes. Symptoms, including chest discomfort in 14 patients and dyspnoea in 11, limited th maximum dose to 8·5 ± 2·3 mg min^{-1} (mean ± SD, 108 ± 24 μg kg^{-1} min^{-1}). At this dose, adenosine reduce pulmonary and systemic vascular resistance (by 38% and 34%, respectively) and increased heart rate (b 34%), stroke index (by 12%) and cardiac index (by 52%). Systemic blood pressure and right atrial pressur did not change. Unexpectedly, adenosine increased left ventricular end-diastolic pressure (LVEDP) (fron 5 ± 6 to 14 ± 10 mmHg, $n=8$), pulmonary capillary wedge pressure (from 3 ± 2 to 10 ± 5 mmHg, $n=16$) an consequently mean pulmonary artery pressure (from 10 ± 2 to 16 ± 5 mmHg). Minute ventilation increased b 84% ($n=11$), resulting in hypocapnia (P_{CO_2}: 31 ± 3 mmHg, $n=8$) and alkalosis (pH: 7·46 ± 0·02, $n=8$) Oxygen consumption was unchanged during the infusion, but increased by 21% 5 min post infusion. All effect were similar in patients with and without coronary artery disease.

Adenosine therefore causes pulmonary and systemic vasodilation and respiratory stimulation. Symptom and an increase in LVEDP of uncertain cause, which occur with high doses, may limit the use of adenosine as systemic vasodilator in conscious subjects. However at lower doses adenosine causes selective pulmonar vasodilation which merits further study.

Introduction

The nucleoside adenosine is a potent vasodilator in many tissues[1–6] and has been used to induce hypotension in patients undergoing intracranial surgery[7,8]. It has been suggested that adenosine might be useful to reduce afterload in patients with heart failure[9]. However, infusion of adenosine into conscious subjects produces effects not seen during anaesthesia, such as marked stimulation of respiration[10], a further action which may have therapeutic application.

Submitted for publication on 20 July 1989, and in revised form 26 January 1990.

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0195-668X/90/111018 + 11 $03.00/0

The haemodynamic effects of adenosine infusion and their relationship to the respiratory changes in conscious subjects have not been fully defined and were therefore the subject of this study.

Methods

We studied 16 patients (11 men), aged 37 to 6 years (mean ± SD: 52 ± 8) and weighing 63 to 95 k (mean 78 ± 10 kg), undergoing cardiac catheter ization for investigation of chest pain. Patients wit unstable angina or heart failure were excluded. W included some patients suspected of having non cardiac chest pain who nevertheless require coronary arteriography: nine patients had norma coronary arteriograms. Exercise stress tests per formed in eight of these patients were negative Although we did not specifically exclude micro vascular angina, all nine were ultimately considere

have non-cardiac pain (musculoskeletal pain: ve, oesophageal spasm: two, hyperventilation syn-rome: two). The other seven patients had coronary rtery disease (two with triple vessel, two with ouble vessel, and three with single vessel disease).

The study was approved by the Hospital Ethics eview Committee (1986) and all patients gave ritten informed consent.

Patients were receiving a variety of medications cluding a β-adrenoceptor antagonist (atenolol) five cases, a calcium antagonist in four, a long-cting nitrate in two, a diuretic in one and amio-arone in one. Treatment with a β-adrenoceptor ntagonist (atenolol) in three further patients had een discontinued 30–45 h previously. All patients ceived diazepam 10 mg orally 1 h before catheter-ation. All had abstained from caffeine-containing everages for at least 6 h.

A pigtail catheter (7F gauge) was inserted rough an 8F sheath in the right femoral artery for easurement of systemic and, in some patients, left ntricular pressures. A balloon tipped catheter wan-Ganz, 7F gauge) was inserted via a 9F sheath the right femoral vein and positioned for easurement of right atrial pressure, pulmonary tery pressure and pulmonary capillary wedge essure (PCWP). The study was then performed llowing an equilibration period of at least 10 min d before any angiograms.

FUSION PROTOCOL

After baseline measurements solutions were fused single blind via the femoral venous eath using a Harvard infusion pump, model 2681. acebo (0·9% sodium chloride) was given for in, followed by a sterile solution (5 mg ml^{-1}) of enosine (Sigma) with dosage increments every in. The initial dose of adenosine, 4·3 mg min^{-1}, d previously been found to produce mild fects[10]. Subsequent doses were 6·1, 8·5, 11·9 mg in^{-1} as determined by the infusion pump. At the d of each stage, patients were asked to report any mptoms and if they were willing to continue to the xt dose.

EASUREMENTS AND SAMPLES

Except as indicated below, measured variables re recorded following the equilibration period seline), during the final 3·5 min of each infusion ge and 5 min following the end of the infusion. e first adenosine stage was continued for 2·5 min or to measurements being made to allow clearing the deadspace of the infusion sheath. We reasoned that steady-state concentrations of adenosine would be achieved quickly because of its short half-life (< 10 s)[11] and because, at the doses used, recirculating venous concentrations are unchanged (unpublished observations).

The electrocardiogram (ECG) was recorded throughout from a single bipolar lead (modified standard lead 2) and used to determine heart rate (HR) and PR interval. Pressures (mean of at least five non-ectopic beats) were recorded via fluid-filled catheters connected to Bell and Howell strain gauge transducers, a Cambridge amplifier, and a Mingograph multichannel recorder (eight patients), or to Hewlett Packard quartz transducers and amplifier and a Gould recorder (eight patients). Patients were supine and the zero reference level was at the sternal angle. Cardiac output (mean of at least three estimations) was measured by the thermodilution technique using an Instrumentation Laboratories computer. Arterial BP was not measured in two patients (numbers 6 and 7) because of a transducer fault. In the last eight patients (three with coronary disease) left ventricular end-diastolic pressure (LVEDP) was also measured at baseline and during the final infusion stage.

Respiration was recorded using a respiratory inductance plethysmograph (Respitrace Corporation Inc.) in the first 12 patients. Isovolume manoeuvres performed by each patient were used to determine the relative contributions of the abdominal and ribcage signals. Volume calibration was then performed using a spirometer (Micro Medical Instruments). A spirometer fault prevented this in the first case. Respiratory rate, tidal volume and minute ventilation were determined from the respiratory trace, recorded using a chart recorder (Rikadenki).

Blood (1 ml) was sampled from the pulmonary artery and aorta at each stage for determination of oxygen saturation (OSM2 Hemoximeter, Radiometer, Copenhagen). Samples (2 ml) were also obtained in the last eight patients at baseline, during the final infusion stage, and 5 min post-infusion, for determination of arterial blood gas tensions and pH (Ciba-Corning Model 178 Blood Gas Analyser).

Derived haemodynamic indices were calculated using standard formulae[12]. Total body oxygen consumption was calculated from arteriovenous oxygen content difference and cardiac output[13].

STATISTICS

Repeated measures analysis of variance and the Student-Newman-Keuls test, or the paired *t*-test,

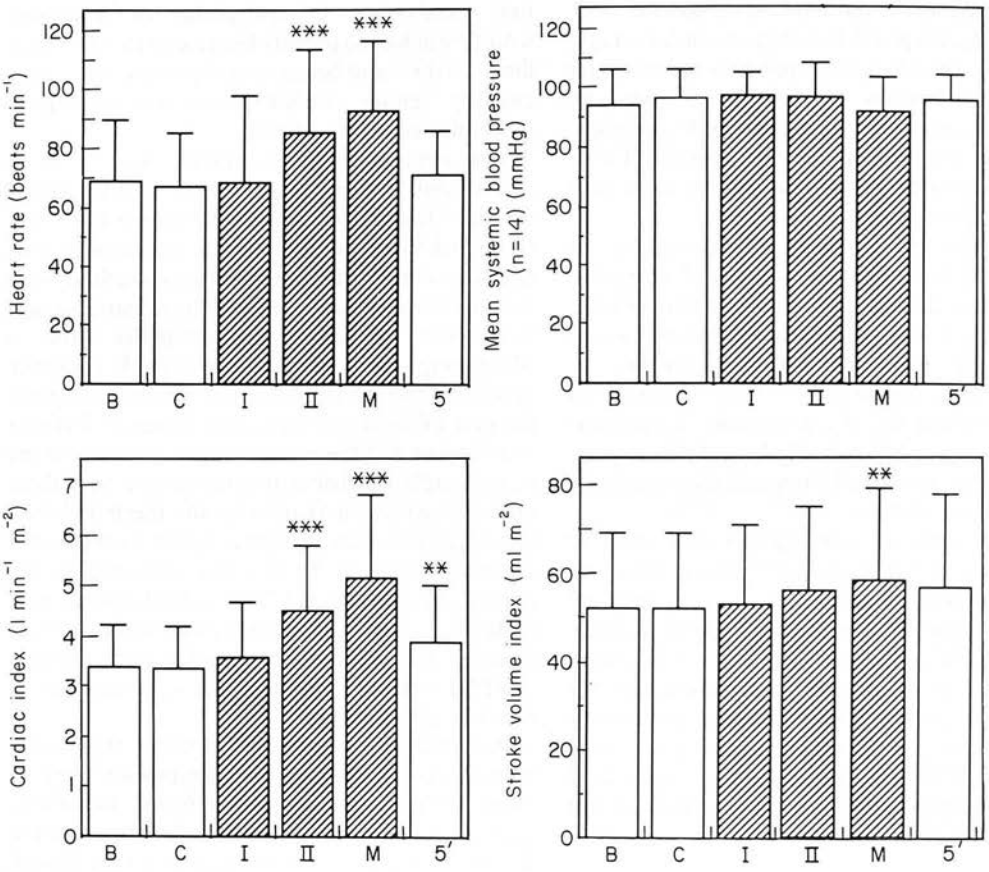

Figure 1 Changes in heart rate, systemic blood pressure, cardiac index and stroke volume index during adenosine infusion. Data are mean ± SD. n = 16 except where shown. B = baseline; C = control infusion; I = 1st infusion stage; II = 2nd infusion stage; M = maximum infusion rate; 5′ = 5 min post infusion. (For explanations of infusion stages please see text.) $*P<0.05$; $**P<0.01$; $***P<0.001$: (for comparisons with baseline).

where appropriate, were used to analyse changes in measured variables. Differences between subgroups according to diagnosis and treatment were compared using the unpaired *t*-test, the Mann-Whitney U-test or the Kruskal-Wallis test for non-parametric analysis of variance, as appropriate. Correlations were examined using the product-moment or Spearman rank correlation coefficients. $P<0.05$ was considered significant.

Results

The maximum dose of adenosine given ranged from 6·1 to 11·9 mg min^{-1} (mean 8·5 ± 2·3 mg min^{-1}, equivalent to 107·9 ± 24·3 μg kg^{-1} min^{-1}) and correlated with both body weight ($r=0.60$, $P<0.02$) and body surface area ($r=0.74$, $P<0.001$). All patients received at least two doses (4·3 and 6·1 mg min^{-1}, equivalent to 56·5 ± 7·8 and 79·3 ± 10·9 μg kg^{-1} min^{-1}).

EFFECTS OF PLACEBO

There were no significant changes during the placebo saline infusion (Figs 1–5, Table 1).

EFFECTS OF ADENOSINE

(a) Lowest dose

At the lowest dose of adenosine, pulmonary vascular resistance fell by 20% (Fig. 3), but there were no other significant haemodynamic changes (Figs 1–3). Minute ventilation, however, was increased by 44% (Fig. 5).

(b) Maximum dose (Table 1)

At the maximum dose, adenosine infusion increased HR by 34% but did not affect systolic,

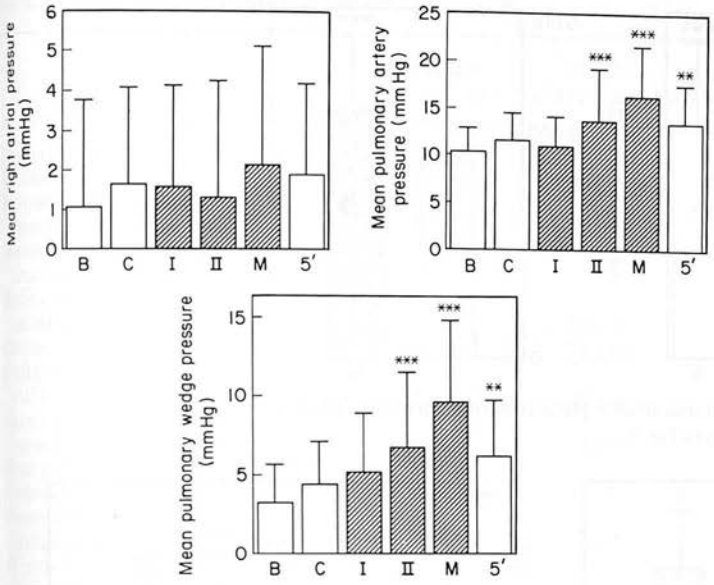

Figure 2 Changes in mean right atrial, pulmonary artery and pulmonary capillary wedge pressures during adenosine infusion. n = 16. Abbreviations as in Fig. 1.

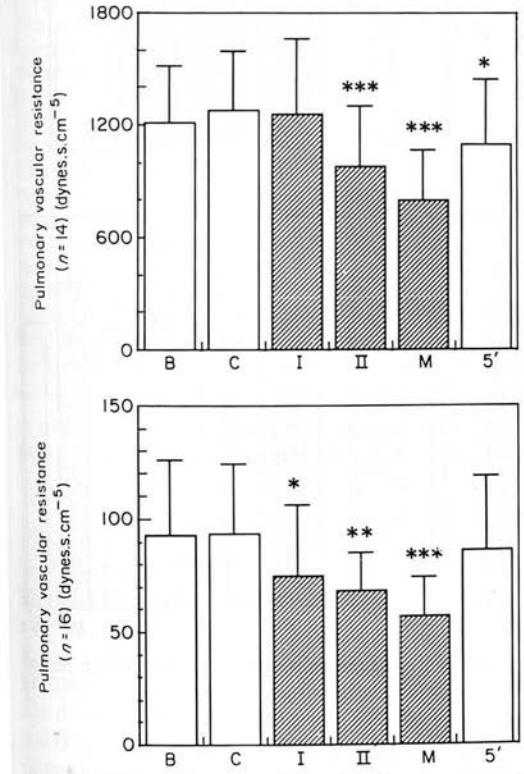

Figure 3 Changes in systemic and pulmonary vascular resistance during adenosine infusion. Abbreviations as in Fig. 1.

diastolic or mean systemic BP (Fig. 1). PR interval was unchanged. Cardiac index increased by 52% and stroke index by 12% (Fig. 1). Mean PCWP and pulmonary artery pressure both increased by 6 ± 5 mmHg to 10 ± 5 and 16 ± 5 mmHg, respectively. Mean right atrial pressure did not change significantly (Fig. 2). Pulmonary and systemic vascular resistance fell, by 38% and 34% respectively (Fig. 3). Total oxygen consumption showed no significant change (Fig. 4). The product of systolic BP and HR, an approximate index of myocardial work, increased by 28% (Fig. 4). LVEDP, measured in eight patients, increased by 9 ± 8 mmHg to 14 ± 10 mmHg. The change in PCWP in this group (3 ± 2 to 9 ± 6 mmHg, $P < 0{\cdot}05$) was similar to the change in the eight patients (four with coronary artery disease) in whom LVEDP was not measured directly (3 ± 3 to 10 ± 5 mmHg, $P < 0{\cdot}001$).

Minute ventilation increased by 84%, due largely to a 60% increase in tidal volume (Fig. 5). The arterial carbon dioxide tension fell by 8 mmHg to 31 ± 3 mmHg and arterial pH increased from $7{\cdot}39 \pm 0{\cdot}03$ to $7{\cdot}45 \pm 0{\cdot}02$. The arterial oxygen tension increased slightly (by 2 mmHg), but this was not statistically significant (Fig. 5).

There was a significant correlation between the percentage increases in heart rate and minute ventilation ($r_s = 0{\cdot}615$, $P < 0{\cdot}05$, n = 12), but neither of these correlated significantly with the percentage

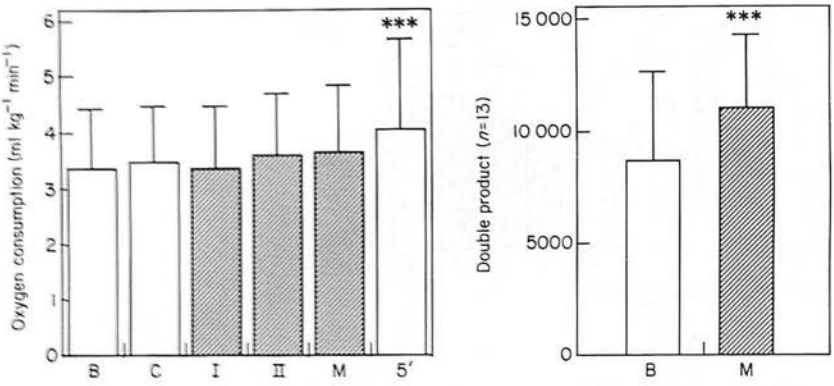

Figure 4 Changes in oxygen consumption and double product during adenosine infusion. n = 16 except where shown. Abbreviations as in Fig. 1.

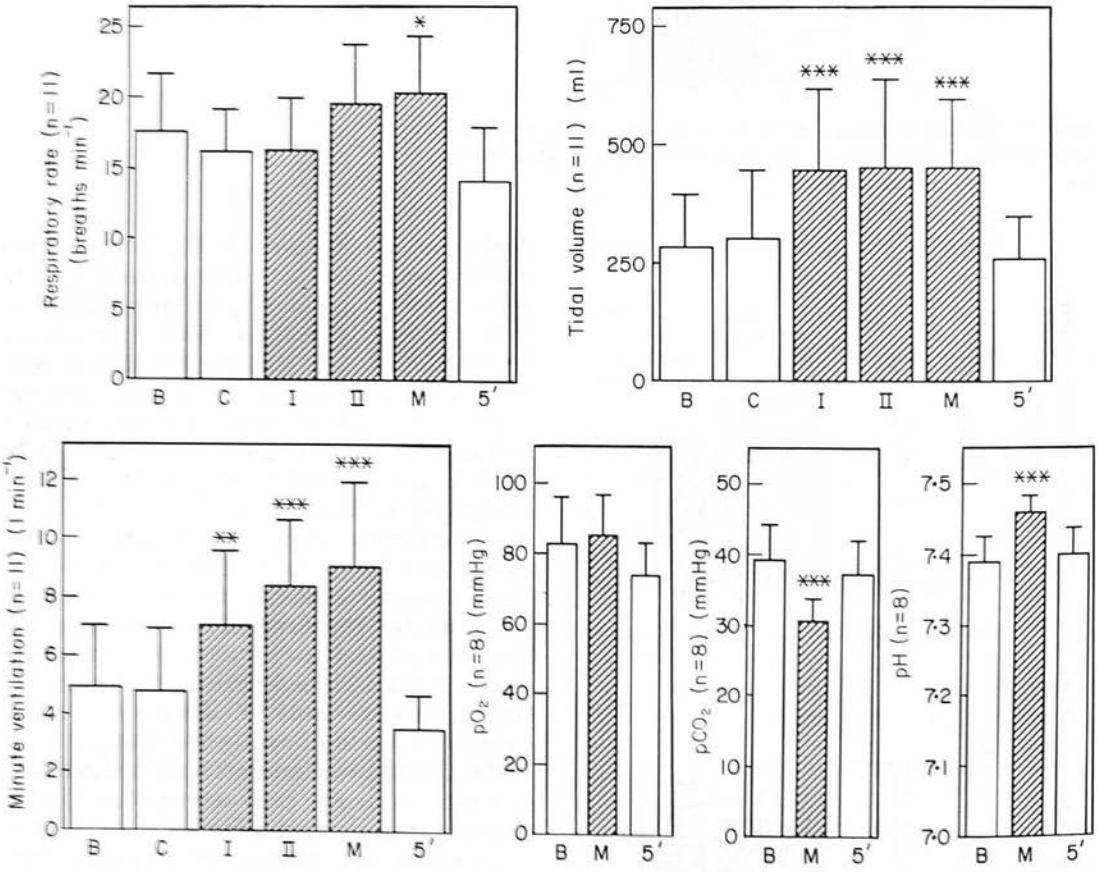

Figure 5 Changes in respiration, blood gas tensions and pH during adenosine infusion. PO_2 = arterial oxygen tension. PCO_2 = arterial carbon dioxide tension. Other abbreviations as in Fig. 1.

decrease in either pulmonary vascular resistance ($r_s = 0{\cdot}165$, n = 16 and $r_s = -0{\cdot}406$, n = 12, respectively) or systemic vascular resistance ($r_s = 0{\cdot}297$, n = 14 and $r_s = -0{\cdot}091$, n = 10, respectively).

(c) Highest dose without symptoms

Ten patients received at least one dose of adenosine without experiencing symptoms. In these patients the maximum dose without symptoms

ble 1 Changes during adenosine infusion at the maximum dose

	Baseline	Placebo	Adenosine	*P**	
	(n = 16 except where indicated)			a	b
art rate (beats min $^{-1}$)	69 ± 21	67 ± 19	92 ± 24	<0·001	<0·001
interval (s) (n = 14)	0·18 ± 0·02	0·18 ± 0·02	0·19 ± 0·02	NS	NS
stolic blood pressure (mmHg) (n = 13)	127 ± 20	134 ± 16	124 ± 16	NS	NS
astolic blood pressure (mmHg) (n = 13)	70 ± 8	73 ± 6	67 ± 8	NS	NS
an blood pressure (mmHg) (n = 14)	94 ± 12	96 ± 9	92 ± 11	NS	NS
rdiac index (l min $^{-1}$ m $^{-2}$)	3·4 ± 0·8	3·3 ± 0·8	5·1 ± 1·7	<0·001	<0·001
oke volume index (ml beat $^{-1}$ m $^{-2}$)	52 ± 17	52 ± 17	58 ± 21	<0·01	<0·001
an right atrial pressure (mmHg)	1 ± 3	2 ± 2	2 ± 3	NS	NS
an pulmonary artery pressure (mmHg)	10 ± 3	12 ± 3	16 ± 5	<0·001	<0·001
an pulmonary capillary wedge pressure (mmHg)	3 ± 2	4 ± 3	10 ± 5	<0·001	<0·001
end-diastolic pressure (mmHg) (n = 8)	5 ± 6	—	14 ± 10	<0·05	—
stemic vascular resistance (dyne.s.cm $^{-5}$) (n = 14)	1209 ± 299	1270 ± 319	794 ± 267	<0·001	<0·001
monary vascular resistance (dyne.s.cm $^{-5}$)	93 ± 32	94 ± 30	58 ± 18	<0·001	<0·001
e pressure product (mmHg.beats min $^{-1}$) (n = 13)	8647 ± 4018	8640 ± 3261	11048 ± 3248	<0·001	<0·001
spiratory rate (breaths min $^{-1}$) (n = 11)	18 ± 4	16 ± 3	20 ± 4	<0·05	<0·01
al volume (ml) (n = 11)	282 ± 113	302 ± 144	452 ± 145	<0·001	<0·001
nute ventilation (l min $^{-1}$) (n = 11)	4·9 ± 2·1	4·8 ± 2·1	9·9 ± 2·9	<0·001	<0·001
gen consumption (ml kg $^{-1}$ m $^{-2}$)	3·4 ± 1·1	3·5 ± 1·0	3·7 ± 1·2	NS	NS
erial PO_2 (mmHg) (n = 8)	83 ± 13	—	85 ± 12	NS	—
erial PCO_2 (mmHg) (n = 8)	39 ± 5	—	31 ± 3	<0·001	—
erial pH (n = 8)	7·39 ± 0·03	—	7·46 ± 0·02	<0·001	—

a are mean ± SD. * *P* values refer to comparisons of values during adenosine infusion with values at baseline (a) and during cebo infusion (b). NS = not significant. LV = left ventricular. PO_2 = oxygen tension. PCO_2 = carbon dioxide tension.

an: 4·7 ± 0·7 mg min $^{-1}$, range: 4·3–6·1 mg n $^{-1}$) caused a 21% fall in pulmonary vascular istance but no other significant changes (Table 2).

MPTOMS

The dose at which symptoms were first reported ied (Fig. 6). Precordial discomfort was reported 14 patients: six of the seven with coronary disease eight of the nine with normal angiography. Ten ents compared the character of the discomfort with their usual chest pain: six (three with coronary disease, three without) thought it was the same and four (two with coronary disease, two without) thought it was different. Two patients who experienced their usual chest pain developed 1-mm ST segment depression. One had normal angiography, the other two-vessel disease.

Dyspnoea was reported by 11 patients and usually first occurred at higher doses than precordial discomfort (Fig. 6). Other symptoms included flushing of the head or neck in six patients

Table 2 Changes during adenosine infusion at the maximum dose given without causing symptoms

	Baseline	Placebo	Adenosine	P*	
	(n = 10 except where indicated)			a	b
Heart rate (beats min^{-1})	61 ± 8	59 ± 7	62 ± 8	NS	NS
Cardiac index ($l\ min^{-1}\ m^{-2}$)	3·4 ± 0·9	3·3 ± 0·8	3·6 ± 1·4	NS	NS
Mean pulmonary artery pressure (mmHg)	10 ± 2	12 ± 3	10 ± 3	NS	NS
Mean pulmonary capillary wedge pressure (mmHg)	3 ± 3	4 ± 3	4 ± 3	NS	NS
Pulmonary vascular resistance (dyne.s.cm^{-5})	96 ± 30	99 ± 28	76 ± 28	<0·05	<0·05
Systemic vascular resistance (dyne.s.cm^{-5}) (n = 9)	1104 ± 280	1173 ± 306	1168 ± 400	NS	NS
Minute ventilation ($l\ min^{-1}$) (n = 8)	5·4 ± 2·4	5·2 ± 2·3	6·7 ± 2·2	NS	NS

Data are mean ± SD. *P values refer to comparisons of values during adenosine infusion with values at baseline (a) and during placebo infusion (b). NS = not significant.

(with another six visibly flushed), headache in four, lightheadedness in three and epigastric discomfort in one.

CHANGES FOLLOWING ADENOSINE INFUSION

Most variables were returning towards baseline values 5 min after the infusion (Figs 1–3). Minute ventilation tended to fall below the baseline value (by 28%) and this was associated with a fall in arterial oxygen tension to 74 ± 9·5 mmHg, but these changes were not statistically significant (Fig. 5). Oxygen consumption increased to 121% of the baseline value 5 min after the infusion ($P < 0·01$) (Fig. 4). Further data in eight subjects (not shown) indicated continuing elevation of oxygen consumption 10 min after the infusion. All symptoms resolved completely within 1–2 min of stopping the infusion.

EFFECTS OF DIAGNOSIS

There were no significant differences in baseline values or changes during adenosine infusion of any variable, between patients with and without coronary artery disease.

EFFECTS OF TREATMENT WITH β-ADRENOCEPTOR ANTAGONISTS

In the three patients whose β-adrenoceptor antagonist treatment, atenolol, was discontinued between 30 and 45 h before the study, baseline HR (93 ± 28 beats min^{-1}) was significantly higher than in those continuing treatment (55 ± 8 beats min^{-1}) ($P < 0·05$), and tended to be higher than in those who had never received such treatment (69 ± 17 beats min^{-1}; NS). There were no other differences in baseline variables between these three groups.

There was a significantly smaller rise in LVEDP, during adenosine infusion, in patients in whom atenolol was recently withdrawn (2 ± 4 mmHg, n = 3) than in those who had not received such a drug (14 ± 6 mmHg, n = 4, $P < 0·05$) (Fig. 7). The adenosine-induced increase in PCWP in those patients in whom atenolol had recently been withdrawn (2·0 ± 4·4 mmHg, n = 3) was likewise smaller, though not significantly so, than in those patients who had never received such a drug (7·9 ± 4·9 mmHg, n = 8) or who continued treatment (6·6 ± 2·6 mmHg, n = 5). There were no other significant differences during adenosine infusion between the three groups. In particular there were no differences in the HR increment between patients not receiving a β-adrenoceptor antagonist, those who were β-blocked and those in whom such treatment had recently been withdrawn (24 ± 9, 21 ± 7 and 26 ± 8 beats min^{-1}, respectively).

Discussion

This study demonstrates that in conscious subjects intravenous infusion of adenosine causes both systemic and pulmonary vasodilation. At the lowest dose used, there was a selective effect on the pulmonary circulation. At higher doses, adenosine also increased left ventricular filling pressure, an effect not previously described.

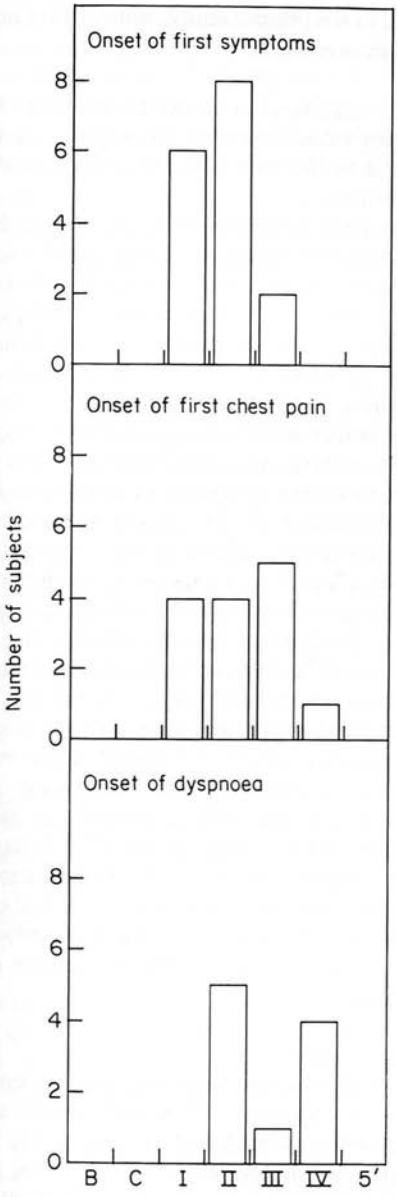

Figure 6 Onset of symptoms during adenosine infusion. III = 3rd infusion stage; IV = 4th infusion stage. Other abbreviations as in Fig. 1.

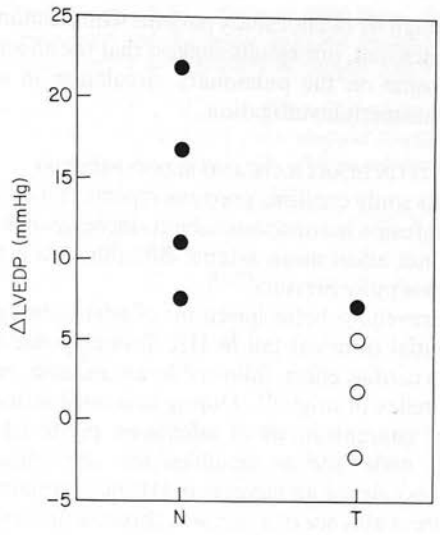

Figure 7 Change in left ventricular end-diastolic pressure during adenosine infusion. Symbols indicate individual subjects. N = not treated with a β-adrenoceptor blocker; T = treated with a β-adrenoceptor blocker (open symbols indicate subjects in whom treatment was discontinued 30–45 h previously).

The haemodynamic effects of adenosine infusion have previously been studied in detail in anaesthetized patients[7,8,14,15]. The main differences observed in this study in conscious subjects were: greater increases in HR and cardiac output, no change in systemic BP and a rise in left ventricular filling pressure. Some patients were receiving cardioactive drugs but, apart from β-adrenoceptor antagonists, these did not appear to affect the responses to adenosine significantly.

EFFECTS ON SYSTEMIC AND PULMONARY VASCULAR RESISTANCE

Although adenosine has been used as a systemic vasodilator during anaesthesia[7,8], the present study shows that in *conscious* subjects infusion rates of adenosine sufficient to reduce peripheral vascular resistance usually cause symptoms. This may limit the clinical use of adenosine as a systemic vasodilator.

The selective effect on the pulmonary circulation at lower doses might be due to rapid clearing of adenosine from plasma during transit through the circulation, since adenosine is avidly taken up by endothelial cells[16] and by red blood cells and has a half-life of < 10 s[11].

Adenosine infusion in the dog caused pulmonary vasodilation during normoxia and prevented the pulmonary pressor response to hypoxia[2]. Recently, Öwall *et al.* reported a 32% fall in pulmonary vascular resistance during adenosine-induced hypotension in anaesthetized patients[14], and McCormack *et al.* reported that adenosine relaxed preconstricted human pulmonary arteries in vitro[17].

Although we did not study patients with pulmonary hypertension, our results suggest that the effects of adenosine on the pulmonary circulation in such patients merit investigation.

EFFECTS ON HEART RATE AND BLOOD PRESSURE

This study confirms previous reports that adenosine infusion in conscious subjects increases HR but does not affect mean arterial BP, although it may increase pulse pressure[10,19].

Intravenous bolus injections of adenosine cause an initial transient fall in HR, probably due to a direct cardiac effect, followed by an increase, probably reflex in origin[18]. During sustained infusion, lower concentrations of adenosine perfusing the sinus node and/or simultaneous activation of reflexes causing an increase in HR may explain the apparent absence of a negative chronotropic effect.

The mechanisms of the increase in HR are not clear. A response to symptoms may contribute. Some but not all workers have shown sympathetic activation during adenosine infusion in conscious subjects[19–21]. There is evidence, however, that the increase in heart rate is mediated predominantly by reduced cardiac vagal tone[22]. This is supported by our finding that the increase was not modulated by treatment with a β-blocker. A major component of the increase may be secondary to the augmentation of ventilation due to carotid body stimulation[23]. The significant correlation we observed between the increases in heart rate and minute ventilation is consistent with this suggestion. Furthermore, injection of adenosine in the aortic arch proximal to the carotid circulation causes an initial increase in heart rate and systolic blood pressure before the onset of peripheral vasodilation, whereas more distal injection does not[20].

That a smaller increase in heart rate is observed in anaesthetized subjects may be partly due to pharmacological or mechanical attenuation of the ventilatory changes, although other factors such as a greater direct negative chronotropic action due to the larger doses of adenosine used or attenuation of baroreceptor effects cannot be excluded. The bigger increase in heart rate in conscious subjects contributes substantially to the larger rise in cardiac index and consequently the unchanged mean blood pressure despite peripheral vasodilation.

Adenosine can also depress conduction in the atrioventricular node[24]. Although a slight increase in mean PR interval and occasional first degree heart block have been observed with adenosine infusion during anaesthesia[14], no such changes were observed in the present study, where lower doses of adenosine were used.

EFFECT ON LEFT VENTRICULAR FILLING PRESSURE

An unexpected finding in this study was an acute increase in PCWP and LVEDP at the higher doses of adenosine.

Ischaemia may have contributed to these changes but they occurred equally in patients with and without coronary artery disease, with and without chest pain (discussed below) and, except in two patients, without ECG changes consistent with ischaemia. The rise in LVEDP might represent the consequence of a negative inotropic effect, consistent with the known ability of adenosine to antagonize both the release of norepinephrine and the β-receptor-mediated responses to catecholamines in the myocardium[25–27]. This might also explain the further observation, albeit in small numbers, that the rise in LVEDP was smaller in patients investigated 30–45 h after stopping atenolol, a time when rebound β-receptor hypersensitivity has been demonstrated[28]. However, it is difficult to implicate a negative inotropic effect when the rise in LVEDP was associated with an increase in stroke output.

Alternatively, adenosine might have reduced ventricular compliance by increasing turgor through its coronary dilator action[1], as has been described in experimental animals[29]. Limitation of such an increase in turgor by hypotension and reduced coronary perfusion pressure could explain the apparent absence of an adenosine-induced increase in left ventricular filling pressure during anaesthesia.

EFFECTS OF RESPIRATION

This study has confirmed a respiratory stimulant property of adenosine[10,30]. Several studies suggest that this effect is mediated at least partly by the peripheral chemoreceptors[31–34]. In this study, ventilation was first increased at a dose of adenosine that caused no haemodynamic changes apart from a fall in pulmonary vascular resistance. Whether adenosine has a physiological rôle in the control of respiration by the carotid bodies, as has been suggested[30], and whether it could be useful clinically as a respiratory stimulant, remain to be shown.

EFFECTS ON OXYGEN CONSUMPTION

The increase in oxygen consumption following adenosine infusion is surprising. During adenosine infusion in anaesthetized subjects, slight falls in whole body oxygen consumption have been

observed, possibly due to shunting of blood away from metabolically active tissues or inhibition of metabolic demands[7,35]. In the present study, such effects might have offset the increased oxygen consumption demanded by the greater cardiac and respiratory work during adenosine infusion. A rebound phenomenon after withdrawal of such mechanisms may have contributed to the increase in oxygen consumption following the infusion.

SYMPTOMS

Symptoms described in this study were similar to those previously reported by healthy volunteers and patients with coronary disease[10,33]. Sylven *et al.* suggested that adenosine release during myocardial ischaemia might mediate the symptom of angina[36]. There is, however, evidence that adenosine may have a more generalized algogenic effect[33,37,38]. This is supported by the present observations that, although in some patients with coronary disease adenosine appeared to reproduce their typical pain, it also did so in some patients thought to have non-cardiac pain, while in others with coronary disease it produced a discomfort different from their angina.

CONCLUSIONS

This study has shown that in conscious subjects, adenosine is a pulmonary and systemic vasodilator and respiratory stimulant, but the dose of adenosine that can be given is limited by the development of symptoms. At high infusion rates, adenosine exerts an adverse effect on left ventricular filling, of uncertain cause. However, at lower rates ($\leqslant 60\ \mu g\ kg^{-1}\ min^{-1}$) adenosine can reduce pulmonary vascular resistance, suggesting that its effects in patients with pulmonary hypertension merit further study.

We are grateful to Dr M. R. Stephens for permission to study patients under his care, to the staff of the Cardiac Catheter Laboratory, University Hospital of Wales for technical support and to the Pharmacy Department, U.H.W., for preparing sterile supplies of adenosine for human use. A.H.W. was supported by the Welsh Scheme for the Development of Health and Social Research.

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